STUDIES ON

VESICULAR-ARBUSCULAR MYCORRHIZAE

IN *ALLIUM* SPECIES

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ABSTRACT

This study is divided into three main parts. The first is an investigation of the effect of vesicular - arbuscular mycorrhizal fungus Endogone on the growth of Allium cepa and Allium porrum.

Onion and leek seedlings were inoculated with Endogone spores and the progression of the mycorrhizal colonisation followed. The effects of the mycorrhizal association on the plants were determined by measuring the shoot and root dry weight, number of roots produced and height of plant. Plants in which mycorrhiza became well established showed significantly enhanced growth rates.

The second part of this study was an investigation on the possible role of vesicular - arbuscular mycorrhiza in the resistance of onion plants to two soil - borne fungal diseases, namely basal root rot, caused by Fusarium oxysporum var. cepa and pink rot, caused by Pyrenochaeta terrestris. A marked resistance to these pathogens was found in plants with a well developed mycorrhizal association. An increased phosphate nutrition of the plants provided by applying $CaHPO_4.2H_2O$ to soil media was found to be insufficient to afford disease resistance.

The third part of this investigation was an attempt to culture Endogone in agar media in the presence of A. cepa, using low phosphate concentrations of $CaHPO_4.2H_2O$,

calcium phytate, sodium phytate or inositol. Germination of Endogone spores in the same phosphate sources containing the vitamins nicotinic acid and thiamine HCl was also investigated. Calcium phytate elicited the best response in the culturing of Endogone in agar media, while the vitamins, used together and in combination with calcium phytate gave the highest percentage germination of Endogone spores.

An ultrastructural study of vesicular - arbuscular mycorrhizal development was carried out to determine the spatial relationships between the endophyte and host cells. Difficulty was encountered with the fragility of the root tissue.

CHAPTER I

INTRODUCTION

1. GENERAL INTRODUCTION

The introduction of the term "rhizosphere" by Hiltner (1905) to signify that region of soil subject to the influence of plant roots has aroused much interest in the relationships between plant and microbe. As a result, there is now a general agreement that in the rhizosphere, microorganisms are more abundant than in soil more distant from the plant. This rhizosphere effect is more pronounced at the "rhizoplane" , the region of soil in the immediate vicinity of the root surface. Bacteria are in greatest abundance, but stimulation of other microorganisms also occurs, that is, actinomycetes, fungi, algae and protozoa.

In the rhizosphere, intense microbial activity occurs between microorganisms as well as between plants and microorganisms, the more specialised microbes often infecting living roots, causing disease. In more specialis--ed fungus-root associations, an intimate mutualistic relationship is established, whereby the plant benefits by being provided with an improved nutrient supply and the fungus by being provided with a medium to reside in and possibly receiving nutrients from the host plant. The host tissue may be modified to an extent (Daft and Okusanya, 1973; Wilhelm, 1973), but this does not necessarily adversely affect the functioning of the root tissue. Frank (1885) first recognised this symbiotic relationship between

tree roots and fungi and introduced the term "mycorrhiza" to denote this unique association. The mycorrhizal state is judged to be a highly specialised form of parasitism (Garrett 1956; Hartig, 1888). This intimate association between plant root and fungus is no longer regarded as exceptional in nature, but rather as the general rule. Three types of mycorrhizae are generally recognised, namely

i. ectomycorrhizae, where the fungus forms an external mantle over the root surface and limited intercellular penetration of the root cortex may occur,

ii. ectendomycorhizae, where the fungus has both external and internal hyphal growth and

iii. endomycorrhizae, where the fungus has limited external hyphae, but very extensive hyphal ramifications within the root cortex. Endomycorrhizae may be further divided into those produced by septate fungi and those produced by aseptate fungi. The present study will be restricted to the latter type, which because of its characteristic morpholo-gical features have been termed vesicular-arbuscular (v.a.) mycorrhizae.

The v. a. type of mycorrhiza is the commonest and most widespread of the mycorrhizae. Endophytes causing these v. a. infections mainly belong to the Phycomycetous genus Endogone. A large proportion of the knowledge on v. a. mycorrhizae has been acquired by using "pot-cultures" in which single Endogone species are maintained on the roots of living plants grown in sterilised soil. The use of pot cultures was first introduced by Mosse (1953, 1956) who inoculated strawberry plants with Endogone spores and

sporocarps she obtained from the roots of other strawberry plants. She obtained typical infections in the newly inoculated plants and new sporocarps formed in the rhizo--sphere of test plants. Following this, many attempts have been made to culture Endogone both in vivo and in vitro (Baylis, 1969; Godfrey, 1957; Hepper and Smith, 1976; Mosse, 1959, 1961; Mosse and Hepper, 1975; Mosse and Phillips, 1971; Powell, 1976; Schenck, Kinloch and Dickson, 1975).

2. AIMS OF THIS INVESTIGATION

The aim of this study is to determine the effect of the v. a. mycorrhizal fungus Endogone on the growth and vigour of onion (Allium cepa) and leek (Allium porrum) plants and to investigate its possible role in the resistance of onions against the soil borne fungal pathogens Fusarium oxysporum var. cepa and Pyrenochaeta terrestris.

CHAPTER II

LITERATURE REVIEW

The study of v. a. mycorrhizae has rapidly expanded. Earlier workers dealt with the occurrence and anatomy of these mycorrhizae and attempted to culture the fungal component. More recent work has centred on the effects of the mycorrhiza on plant growth and phosphate uptake and in the fungi themselves, especially their ecology and taxonomy. This change in interest may be attributed to various factors, among them the determination of the identity of v.a. endophytes, replacement of impure inocula with purer inocula and the development of improved techniques for the isolation of the endophyte and for the study of infected root tissue.

1. MORPHOLOGY OF ENDOGONE

The morphology of the mycelium external to the host root is different from that internally.

1.1. External morphology

The amount of external hyphae varies considerably from root to root. It may be almost absent, present as a few strands of hyphae or may form localised masses of hyphal network. Although hyphae may occur along the root surface, the mycelium has a limited spread (up to 1 cm.) from the root and can be traced into the root. The hyphae are very characteristically dimorphic (Mosse, 1959; Nicolson, 1959, 1963), irregular, thick walled and aseptate.

Arising from angular projections of these thick walled hyphae are vesicles, thick walled spores and smaller, thin walled hyphae (Gerdemann, 1968). Nicolson (1967) suggested that these thin walled hyphae are ephemeral, being aseptate initially, but becoming septate as they lose their contents. On degradation, Nicolson (1959) suggests that these hyphae leave behind the characteristic projections seen on the main aseptate hyphae. Powell (1976) found septate , pre-infection, fan like structures arising from Endogone spores in very close proximity to onion roots. He suggested that these may be sites of cytological changes that are necessary before hyphae become "physiologically infective" and during later stages, these pre-infection structures undergo cytoplasmic withdrawal and slough of to become the angular projections described by Nicolson (1959).

1.2. Internal morphology

The mycorrhiza formed by the Phycomycete Endogone has two very diagnostic features, namely vesicles and arbuscules. The functions of these structures have come under considerable debate with many conflicting opinions.

The fungus first forms an appressorium on the root surface and then an infection peg penetrates an epidermal cell behind the meristematic region. Hyphae then spread intra- and intercellarly in the cortex. They are irregular and variable in shape and size, forming complex coils, loops and anastomoses (Mosse, 1956). After the fungus is well established in the root cortex, arbuscules are formed intracellarly. These develop by repeated dichotomous

branching from one or several intracellular hyphae which arise as short lateral branches from distributive hyphae, resulting in a complex structure, not unlike a bunch of grapes, which may occupy the entire lumen of the cell. At maturity, these arbuscules collapse and in so doing, are thought to release nutritive material to the plant. Intra- and intercellar vesicles develop later. These are terminal swellings of distributive hyphae, whose size and shape depend on the host type and its nutritional status(Mosse, 1963). Older vesicles have vacuoles and oil droplets in their cytoplasm. In the older cortex, vesicles may become so abundant that they protrude from the root (Mosse, 1963) or distort root structure (Koch, 1935). Vesicles are believed to be storage and reproductive in function. The accummulation of oil suggests a storage function (Mosse, 1963). In mature vesicles, these oil droplets coalesce to form one large droplet surrounded by a thin peripheral cytoplasm. In their reproductive functions, vesicles are thought to act either as reproductive propagules following decay of host roots (Mosse, 1963; Schrader, 1958) or as sporangia from which small endogenously produced spores are liberated (Koch, 1961; McClennan, 1926; Otto, 1954). Koch (1961) and Otto (1959) thus postulated that young vesicles served as storage organs while older ones act as over-wintering and reproductive structures following root decay.

2. ECOLOGY OF ENDOGONE

2.1. Occurence

Among the many fungi that inhabit plant roots or colonise the rhizosphere, the v. a. type is by far the most

widespread. It is world wide in its distribution, occuring on plants from the tropics (Janse, 1897; Johnson, 1949) to the Arctic (Katenin, 1963). They occur in most habitats, except in aquatic environments and in plants that grow in wet conditions, for example, rice (Asai, 1934). These fungi have an extremely wide host range, forming associations with Bryophytes, Pteridophytes, Gymnosperms and Angiosperms. Butler (1939) reviewed the occurrence of v. a. mycorrhizal association in fossil plants. It is most unusual for obligately parasitic fungi to have such a wide host range. Apparently, most plants are unable to prevent the establishment of this type of association.

V. a. mycorrhizae are the predominant mycorrhizal types present in the main crop plants which are of great economic importance. Their abundance led Wilhelm (1966) to state "... under agricultural field conditions, crops do not, strictly speaking have roots, they have mycorrhizae". The Gramineae (Asai, 1934; Gerdemann, 1964, 1965; Khan, 1972; Nicolson, 1959, 1960; Saif and Khan, 1975; Sutton, 1973) and Leguminosae (Jones, 1924; Samuel, 1926) are among the families of economic importance in which v. a. mycorrhizae occur. Among the important crops that have been reported to have this mycorrhizae are the Allium family (Mosse, 1961, 1972; Hayman, 1974; Hayman and Mosse, 1971, 1972; Zink, 1962, 1963a, 1965), apple (Mosse, 1957), barley (Benians and Barber, 1972), beans (Sutton, 1973), cacao (Laycock, 1945), citrus (Hattingh and Gerdemann, 1975; Kleinschmidt and Gerdemann, 1972; Marx, Bryan and Cambell, 1971), coconut (Johnston, 1949), coffee (Janse, 1897),

cotton (Bird, Rich and Glover, 1972; Rich and Bird, 1974), grape (Deal, Boothroyd and Mai, 1972; Possingham and Obbink, 1971), legumes (Daft and El-Giahmi, 1974,1975; Jackson, Franklin and Miller, 1972; Mosse, 1972; Ross, 1971; Ross and Gilliam, 1973; Ross and Harper, 1970; Samuel, 1926), maize (Gerdemann, 1964; Khan, 1972; Sutton, 1973), oats (Meloh, 1963), pea (Jones, 1924; Sutton, 1973), potato (Hayman, 1970), rubber (Wastie, 1965), strawberry (Holevas, 1966; Mason, 1964; Mosse, 1956), tobacco (Koch, 1935; Peuss, 1958), tomato (Daft and Nicolson, 1969, 1972; Daft and Okusanya, 1973) and wheat (Hayman, 1970; Khan, 1973; Saif and Khan, 1975).

2.2. Recovery of Endogone spores

The near universal occurrence and abundance of Endogone v. a. mycorrhizae has been brought to the fore by the development of efficient methods of recovering Endogone spores from the soil. Thaxter (1922) first reported collecting spores by picking them up as large sporocarps or spore masses either on or beneath the soil, or from mosses or organic litter. Barrett (1947, 1961) used a hemp seed technique and isolated eleven species of fungi from mycorrhizal roots. Dowding (1955) found large spores from the alimentary tracts of rodents at autopsy.

Endogone spores are usually recovered from soil by the wet sieving and decanting procedure of Gerdemann (1955). This technique, originally employed by nematologists to collect eelworms, was modified by Gerdemann to separate an organic fraction, which included Endogone spores, from heavier soil particles. Other techniques developed include

those by Mosse and Jones (1968) who used a differential sedimentation method on gelatine columns; by Ohms (1957), who employed a floatation method; by Ross and Harper (1970) who used a sucrose density gradient column method; and by Sutton and Barron (1972) who developed an adhesion-floatation technique.

The ease by which mycorrhizal roots may now be examined has contributed to the rapid progress in the study of v. a. mycorrhizae. Roots are merely cleared with KOH, appropriately stained and the endophytes are then readily visible under a microscope (Bevege, 1968; Phillips and Hayman, 1970)

2.3. Effect of environmental factors on Endogone colonisation and spore populations

Studies have been made on the effect of various factors on Endogone colonisation and spore populations. Considerable seasonal fluctuations in spore numbers have been reported (Hayman, 1970; Mejstrik, 1972; Redhead, 1971; Saif and Khan, 1975; Sutton and Barron, 1972). There is agreement that spore populations generally increase during seasons of slow root growth (Mason, 1964; Saif and Khan, 1975) and when root growth is intermittent (Mosse and Bowen, 1968). Plants growing in very wet or water-logged conditions show significantly reduced spore numbers(Asai, 1934; Kessler and Blank, 1972), however, the situation is reversed if the water table becomes lower (Mejstrik, 1965) or if the plants are transplanted to well drained soils (Maeda, 1954). Heavy shading is reported to reduce spore numbers significantly (Bevege, 1972). There is good evidence that

large applications of fertilisers reduce spore formation (Hayman, 1970, 1975; Krucklemann, 1975; Mosse and Jones, 1968; Porter and Beute, 1972)

It has been suggested from various surveys (among others, Daft and Nicolson, 1972; Hayman, 1970) that the degree of Endogone colonisation may be indicated by the number of external spores that are visible under a binocular microscope. The extent of colonisation can vary considerably from only a few roots to the whole of the root system. The degree of colonisation is difficult to ascertain, but various means have been employed to estimate the extent of mycorrhizal development. An approximate grading may be effected by visual examination of the root system, as some mycorrhizal roots tend to be discoloured, for example, yellow in the onion (Mosse, 1972) and maize and tomato (Daft and Nicolson, 1966). This visual method of estimating the extent of mycorrhizal development is limited because only a few plant species are so effected. The most widely used means of estimation of v. a. mycorrhizal development is that where roots are cleared by KOH, stained with lactophenol blue and then examined under the microscope for the presence of v. a. endophytes. Other methods include finding external mycelia and external spores and accurate recordings of the abundance of vesicles and arbuscules (Hayman, 1974; Mosse and Phillips, 1971; Saif and Khan, 1975)

Two main factors, light intensity and nutrient availability, have been shown to influence the prevalence

of endomycorrhizae. Difficulty in maintaining vigorous mycorrhizal association in glasshouse grown plants were thought to be due to insufficient light (Peyronel, 1940; Stahl, 1949). Peuss (1958) found that a 50% decrease in light intensity reduced colonisation by two-thirds in tobacco. Schrader (1958) reported similar effects on pea seedlings. Most workers have attributed the reduction in mycorrhizal colonisation to a deficiency in the supply of assimilation products to the roots. Plant growth is also adversely affected under low light conditions, which may be attributed to the fungus draining the host's already depleted carbohydrate supply. Hayman (1974) suggested that the poor light conditions cause a deficiency in functional arbuscules, thus not supplying enough phosphate for plant growth in soils deficient in phosphate, rather than the host being starved of photosynthate.

There is evidence that fertiliser treatment can adversely affect v. a. mycorrhizal development. Such reports have been made, for example, in Coprosma robusta (Baylis, 1967), in tomato, tobacco and maize (Daft and Nicolson, 1966) and in barley, oats, rye and wheat (Strzemska, 1975).

2.4. Effect of Endogone on plant nutrition

Research in the last decade has established that v. a. mycorrhizae can be formed by a wide range of fungi which differ, not only taxonomically, but also in their ability to enhance phosphate uptake of plants and to promote plant growth. Such work has been carried out in various media, for example, in sand (Daft and Nicolson, 1969) in soil (Baylis, 1959, 1967; Clark, 1963, 1964; Gerdemann,

1964; Mosse, 1957), in water (Peuss, 1958) and in plant and soil culture (Bevege, 1970). These studies have usually been conducted under controlled environmental conditions, but a few experiments have been conducted in the field (Khan, 1972, 1973; Ross, 1971).

2.4.1. Effect of Endogone on plant phosphate uptake

Many earlier workers, for example, Baylis (1959) and Gerdemann (1964) have proposed that the improved growth of mycorrhizal plants may be attributed to increased phosphate uptake. Recent research has seen heavy emphasis being laid on the phosphate uptake aspect in studies concerning the effects of v. a. mycorrhizae on plant growth.

There is evidence to show that many plant species growing in soils containing little available phosphate tend to increase their uptake of phosphate if colonised by v. a. mycorrhizae. Among the plant species that show this are the tropical legume Centrosema pubescens (Mosse, Hayman and Arnold, 1973), citrus (Kleinschmidt and Gerdemann, 1972), Griselina (Baylis, 1959), maize (Khan, 1972), onion (Hayman and Mosse, 1971), soya bean (Ross and Gilliam, 1973), strawberry (Holevas, 1966) and wheat (Khan, 1973). The plant responses to v. a. mycorrhiza varied considerably with different species and sub-species, as well as with the levels of available phosphate. There is general agreement that low levels of available phosphate elicit a greater increase in phosphate uptake.

Considerable work has been done on the effects of v. a. mycorrhizae on plants supplied with phosphate. The

addition of soluble phosphate improved the growth of non-mycorrhizal plants more than that of mycorrhizal plants (Baylis, 1967; Daft and Nicolson, 1966, 1969; Holevas, 1966; Khan, 1972, 1973; Mosse, 1973; Murdoch, Jackobs and Gerdemann, 1967). Correspondingly, there have been reports of a reduction in mycorrhizal development with the addition of phosphate (Baylis, 1967, 1970; Daft and Nicolson, 1969; Khan, 1972, 1973; Mosse, 1973; Ross, 1971). Mosse (1972) reported that with the addition of more than 0.2g. of $Ca(H_2PO_4)_2$ per kg. of soil, mycorrhizal plants showed slower growth rates than non-mycorrhizal plants. This was probably due to phosphate toxicity, resulting from supra-optimal phosphorus concentrations being reached sooner by the increased uptake of phosphate in mycorrhizal plants.

Studies have been made to test the ability of mycorrhizal plants to utilise phosphate sources of different availability when supplied to growth media. Daft and Nicolson (1966) found that the improved growth rates of mycorrhizal tomato plants with small additions of bonemeal (containing phosphorus) decreased when as much as sixteen times bonemeal was supplied instead. These workers also found that the sparingly soluble tricalcium phosphate produced far greater growth than the more soluble di-calcium phosphate and finely ground apatite. Murdoch, Jackobs and Gerdemann (1967) found similar results with tri-calcium phosphate and to a lesser extent, with rock phosphate, compared to the more soluble monocalcium phosphate and superphosphate. From these results, it was proposed that v. a. mycorrhizae enabled plants to utilise sources of phosphate that were generally less soluble or less available

to non-mycorrhizal plants.

The employment of isotopically labelled soils and nutrient solutions clarified the situation. Using phosphorus - 32 labelled solutions, Bowen and Rovira (1968), Gray and Gerdemann (1967) and Morrison and English (1967) confirmed that mycorrhizal roots take up more phosphate than non-mycorrhizal roots. Subsequent experiments using phosphorus - 32 labelled solutions (Hattingh, Gray and Gerdemann, 1973; Mosse, Hayman and Arnold, 1973) and phosphate labelled soils Benians and Barber, 1972; Hayman and Mosse, 1972; Sanders and Tinker, 1971) showed the specificity of phosphorus in mycorrhizal and non-mycorrhizal plants were very similar, indicating that contrary to the earlier hypothesis, both mycorrhizal and non-mycorrhizal plants utilise similar fractions of phosphate in growth media and that v. a. mycorrhizae do not enable mycorrhizal plants to utilise generally unavailable sources of phosphate. It was concluded from these experiments that v. a. mycorrhizae do not mobilise soil phosphate, but greatly increase the utilisation of available phosphate (Mosse 1973a; Sanders and Tinker, 1971; Tinker, 1975). It is now accepted that the mycelial network of v. a. mycorrhizal fungi enables plants to remove phosphate from a larger soil volume, extending beyond the immediate vicinity of the root surface. Calculations by Bieleski (1973) indicate that within a 1mm. length of root, four hyphae, each extending twenty mm. from the root surface, would increase phosphate uptake by sixty times, if diffusion was limiting and ten times, if uptake was proportional to surface area, greater

than that possible in a non-mycorrhizal plant.

2.4.2. Effect of Endogone on the uptake of other nutrients. V. a. mycorrhizae have also been shown to affect the uptake of nutrients other than phosphate by plants. Mycorrhizal plants were found to contain higher concentrations of copper (Mosse, 1973; Ross, 1971; Ross and Harper, 1970), calcium and nitrogen (Ross, 1971; Ross and Harper, 1970), magnesium (Mosse, 1973; Holevas, 1966), manganese (Mosse, 1973; Ross and Harper, 1970), strontium (Jackson, Miller and Franklin, 1973) and zinc (Mosse, 1973). Mycorrhizal plants tend to have lower concentrations of potassium (Holevas, 1966; Mosse, 1973). The uptake of other compounds may also be increased, for example, that of dihydrostreptomycin has been found to be greater in mycorrhizal maize (Meloh, 1963).

2.5. Inoculation of plants with Endogone

A variety of techniques have been developed for the inoculation of plants with Endogone. Many workers have used surface sterilised mycorrhizal roots of field grown maize plants as the inoculum. Clark (1963) employed this technique of inoculating in yellow poplar grown in soils fumigated with methyl bromide. Meloh (1963) used this method on oats and maize, as did Murdoch, Jackobs and Gerdemann (1967) on sudan grass. Similarly, Winter and Meloh (1958) employed this means of inoculation on maize. Jackson, Franklin and Miller (1972) inoculated maize, soybean and sudan grass using lyophylised, ground mycorrhizal roots. Peuss (1958) inoculated tobacco plants by grafting a piece of infected cortical tissue in the tap root. All these workers reported that mycorrhizal plants grew better than non-mycorrhizal

ones.

Baylis (1959) found that Griselina littoralis seedlings grown in soil containing the fungus Endogone was a successful means of establishing v. a. mycorrhiza development. Using the same technique, Baylis, McNabb and Morrison (1963) observed that inoculated two year old Podocarpus totara which became mycorrhizal were seven times heavier than control plants. Ross and Harper (1970) obtained inocula for soybean plants grown in fumigated plots by culturing Endogone monoxenically on soybean seedlings in jars covered by a thin laboratory film (Parafilm M-6).

The employment of purer inocula consisting of sterilised Endogone spores or sporocarps or sterilised soil inoculated with these in the presence of a host plant are now being employed, after the establishment of the "pot culture" technique by Mosse (1953). She discovered a species of Endogone in association with a glasshouse grown strawberry plant and upon experimentation, she demonstrated that the species could be maintained on plants grown in open pots. Coupled with Gerdemann's (1955) method of wet sieving for recovering Endogone spores, pure inocula in the form of spores or sporocarps may now be produced and maintained effectively.

2.6. Interactions between Endogone and other microorganisms

The increase in awareness of v. a. mycorrhizae in plants has resulted in greater attention being drawn to the

effects of the interactions between Endogone and plants, but little attention has been paid to the interactions between Endogone and other microorganisms. This seems peculiar for the reason that as Endogone is involved both internally and externally with the root, Endogone must be present in the region termed the "rhizoplane", that is, the region of the most intense microbial interactions in the rhizosphere. Presumably , the result of the sum of these interactions are mainly not deleterious to plant growth, as most reports of the effects of v. a. associations have been favourable. Nevertheless, this raises the possibility that any effect reported, be it beneficial or deleterious to plant growth, may in fact reflect not just the influence of Endogone alone but an interaction between Endogone and other microorganisms.

A detailed study of the rhizosphere effect, which would include interactions between soil microbes and between plant and microbes, would be difficult to conduct, especially in simulating natural environments. Some investigations have been conducted on the interactions between Endogone and other microorganisms. Asai (1948) reported the necessary presence of v. a. mycorrhiza for effective nodulation in many legumes. The improved nutritional status of the mycorrhizal plants has been said to have increased the number of tobacco mosaic virus lesions (Schonbeck and Schinzer, 1972) and to have increased the amount of extractable tomato aucuba, potato X and Arabis mosaic virus (Daft and Okusanya, 1973). Conversely, Baltruschat and Schonbeck (1972) found that mycorrhizal

tobacco plants were less susceptible to Thielaviopsis basicola than non-mycorrhizal ones and that an extract of mycorrhizal tobacco roots strongly inhibited formation of chlamydospores of T. basicola in vitro. The presence of v. a. mycorrhiza has also been thought to have an adverse effect on root knot nematode development (Baltruschat, Sikora and Schonbeck, 1973; Schenck, Kinloch and Dickson, 1974).

One interaction that has received very little attention is that between v. a. mycorrhizae and plant disease causing microorganisms. Improved nutrition by itself is insufficient explanation for the beneficial effect v. a. mycorrhizae have on plant growth. Other factors need to be considered, especially the role of v. a. mycorrhizae in plant disease resistance. Protection against disease may be afforded by the mycorrhizae by providing a physical barrier, secreting antibiotics, secreting exudates that favour protective rhizosphere organisms, or perhaps by utilising surplus carbohydrates, thus reducing the attractiveness of the root to pathogens (Zak, 1964). Little attention however, has been paid to the role of v. a. mycorrhizae in plant disease, compared to that received by ectomycorrhizae. Ectomycorrhizae have been shown to be antagonistic, among others, to Phytophthora cinnamomi (Bryan, 1960; Bergemann, 1956), to Cenococcum graniforme (Bergemann, 1956) and to Fomes annosus (Rypacek, 1963) on pine trees.

2.7. Fusarium oxysporum var. cepa and Pyrenochaeta terrestris-the pathogens under study.

F. oxysporum v. cepa (Snyder and Hansen, 1940), which causes basal root rot of onion, has been recorded in various parts of the world, for example, North America, South America, South Africa, Australia, Japan and the Philippine Islands. In New Zealand, it has been reported at various growing centres, especially in the Pukekohe area (Dingley, 1961). Some specimens have been obtained at the Katikati (Bay of Plenty) area. The disease is characterised by diseased plants being infected only on one side of the bulb, where lesions are formed, which destroys the basal portion of the bulb and a high proportion of the root system. This peculiar one-sided infection has earned it the common name of "leaners". It is often associated with the onion maggot and is of considerable economic importance, particularly in the Pukekohe area. The Marshlands area in Christchurch where advice was sought from some onion growers, is relatively free from the disease.

Pyrenochaeta terrestris (Gorenz, Walker and Larsen, 1948) which causes pink rot of onion has been described to be common in onion crops throughout New Zealand (Dingley, 1965), particularly in an onion crop grown on land recently brought into cultivation from pastures. In other countries, it has also been reported present on grass roots (Dingley, 1969). Pink rot of onion was originally thought to have been caused by Fusarium malli by Taubenhaus and Mally (1921) but later thought to be caused by Phoma terrestris (Hansen, 1929). Following the discovery of bristles (setae) on the

external surface of the pycnidium, it was transferred to the genus Pyrenochaeta by Gorenz, Walker and Larsen in 1948. As suggested by its name, onions infected by the disease exhibit characteristic pink coloured roots, with retarded bulb growth. It is not uncommon in the Pukekohe area. The Marshlands is again relatively free from the disease.

2.8. Culture of v.a. mycorrhizal fungi.

Various attempts have been made to culture v. a. mycorrhizal fungi. Jones (1924) obtained limited growth of the endophyte, but found that it could not be subcultured successfully. Magrou (1936) first successfully synthesized v. a. mycorrhiza in agar by placing a non-mycorrhizal Arum maculatum plant near a mycorrhizal root segment.

The employment of spores in attempts to isolate Endogone has not been very successful. This was either because spore germination failed (Godfrey, 1957) or when germination occassionally occurred and hyphal growth was considerable, the fungi could not be subsequently subcultured (Gerdemann, 1955; Godfrey, 1957, Mosse, 1959, 1962).

Using a hemp-seed baiting technique, Barrett (1961) claimed to have isolated a true endophyte, but using isolates obtained from Barrett, workers such as Gerdemann (1968) and Mosse (1962,1963) found inconsistent results, especially in establishing infection in plants grown under aseptic conditions using sterilised inocula. Mosse (1962) found that pre-germinated, surface-sterilised spores of an Endogone species may cause infection in clover seedlings

only in the presence of bacteria (a pseudomonad) or an extract from a bacterial culture. Recently, v. a. mycorrhizae have been grown in pure culture on agar media using both pre-germinated Endogone spores on Trifolium parviflorum, T. pratense and tomato seedlings (Mosse and Hepper, 1974) and ungerminated Endogone spores on T. parviflorum seedlings (Mosse and Phillips, 1971). Schenck, Graham and Green (1975) found high germination rates (upto 95%) of several Endogone species, using Mosse's medium 16 (as in Mosse and Phillips, 1971) or soil extract agar. Similarly, Hepper and Smith (1976) obtained over 90% germination of Endogone mosseae spores on distilled water agar. Using water, nutrient and soil extract agar, Daniels and Graham (1976) found that an excessive nutrient supply inhibited germination of Endogone spores. They suggested that thefactors that favour spore germination are present as natural components in some of the agars and can be washed from the agar. They believed that extracts that are autoclaved, chloropicrin-treated or soil that is air-dried are prone to nutrient release in amounts large enough to suppress germination of Endogone spores. Powell (1976) found that Endogone mosseae spores germinated readily on agar coated glass slides buried in soil both in the presence and absence of Allium cepa seedlings.

2.9. Microscopic studies of v. a. mycorrhizae development

Light microscopic studies by earlier workers such as Janse (1897), Gallaud (1905), Kessler (1966), McClennan (1926) and McLuckie and Burgess (1932) have revealed the anatomy, development and distribution of the v. a.

endophyte. Following these workers, other descriptions have been made by more recent workers, notably Mosse and Hayman.

The limited depth of field and resolution and restricted determination of the three dimensional morphology afforded by the light microscope had not enabled the spatial relationships between host cell and v. a. endophyte to be elucidated. Thus, ultrastructural techniques were developed to clarify such relationships. The earliest reported work in such studies is probably that by Becking (1965). Since then, considerable interest have been generated involving scanning electron microscopic studies (Kinden and Brown, 1974, 1975, 1976; Schoknecht and Hattingh 1976) and transmission electron microscopic studies (Cox and Sanders, 1974; Kinden and Brown, 1975; Nieuwdrop, 1972). These workers have presented a clearer picture of the relationships between host cells and vesicular-arbuscular mycorrhizal endophytes and notably, clarified the structure of the finer, terminal branches of arbuscules.

CHAPTER III

MATERIALS AND METHODS

1. ISOLATION OF ENDOGONE SPORES

1.1. General methods

In the employment of the wet-sieving and decanting technique of Gerdemann (1955) in the isolation of Endogone spores, the soil is washed through a series of sieves with decreasing pore diameter. The spore containing fraction thus recovered is then spread on "Dicel" cloth and counted under a binocular microscope (Hayman, 1970; Mosse and Jones, 1968). But the resulting sievings containing the spores are accompanied by a large amount of organic debris, which makes quantitative estimations very difficult. The wet-sieving procedure is also very time consuming, restricting the number of soil samples that may be processed and makes statistical comparisons difficult (Mason, 1964; Nicolson, 1967). Attempts have been made to separate the spores from the debris collected on the sieves. Ohms (1957) centrifuged the organic fraction on a sucrose gradient, but the 5% sucrose needed to effectively separate the spores from the debris damaged the spores (Mosse and Jones, 1968). Mosse and Jones (1968) attempted to improve spore separation by using a technique of sedimentation on layered gelatine columns which have smaller osmotic pressure than 5% sucrose. This method is also time consuming and the gelatine concentrations and sedimentation times need to be very carefully adjusted according to soil type. Ross and Harper (1970) used a sucrose density column method which was

probably a modification of Ohm's (1957) method. Sutton and Barron (1972) developed a floatation-adhesion technique which depended on the adhesion of Endogone spores to a glass surface at the meniscus. This technique gave a 94 to 98% recovery, the highest recovery rate described to date. In this study, this technique was employed to effectively isolate Endogone spores.

1.2. Soils

Soil was obtained from fields in the vegetable growing area of Marshlands near Christchurch. These fields had been in onion monoculture, so that the Endogone spores recovered were more likely to have been of a uniform type. Samples were collected from fields where onions had been planted in spring and autumn to determine whether the difference in times of planting the host plant affected the Endogone spore populations at sampling times, that is, July and October 1974 and January and March 1975.

Soil samples were taken at various depths between 5 and 40 cm. and placed in plastic bags which were stored at 5°C until spore isolations were made 1 to 2 days later.

1.3. Technique employed in Endogone spore isolation

Spore recovery was accomplished by employing the technique developed by Sutton and Barron (1972), which depended on the adhesion, rapid floatation and hydrophobic properties of Endogone spores. A 100 g. fresh weight of soil was placed in a 500 ml. beaker half filled with distilledwater and gently agitated and fragmented with a glass rod to obtain a soil suspension. This was left for 5

minutes to allow the soil suspension to settle. The Endogone spores tended to float and accumulated in the scum of the margin of the meniscus. Large pieces of debris were removed. The soil suspension was then slowly decanted into a 250 ml. separating funnel, thereby collecting the scum layer which contained the Endogone spores. More water was added to the soil remaining in the beaker and the decanting procedure repeated twice to ensure recovery of any remaining Endogone spores. The suspension in the funnel was allowed to settle for 5 minutes to allow the scum layer to form at the margin of the meniscus. The water was then drained from the funnel at a rate of 80-100 ml. per minute into another funnel. After 5 minutes, this was similarly drained and the spore-free water collected for later use. A funnel, supporting a 9 cm. disc of filter paper, was placed in a 250 ml. flask which was in turn placed beneath one of the separating funnels. Endogone spores along with other scum particles were then gently washed from the inner walls of the separating funnel with distilled water onto the filter paper. Care was taken to prevent overflowing of the funnel supporting the filter paper and thus the possible loss of Endogone spores. In addition, larger particles of debris tended to block up the bottom orifice of the separating funnel and had to be frequently prodded along with a piece of wire. Another funnel supporting a filter paper disc was placed under the second separating funnel and Endogone spores similarly collected. A photograph of the experimental set-up is provided (Figure 1). Several subsamples may be processed simultaneously.

When all the visible particles in the separating funnels were transferred onto the filter papers, the latter were each placed in a petri dish. These were examined under a binocular microscope at 40 X magnification and the Endogone spores identified and counted. The spores were then stored in petri dishes at 5°C for 6 weeks before use.

2. INOCULATING PLANTS WITH ENDOGONE SPORES

Numerous methods have been employed in attempts to establish v.a. mycorrhiza in host plants. These methods have been surpassed in favour of that using a pure inoculum of Endogone spores (Mosse, 1953). In this study, a method was devised whereby Endogone spores collected as described previously, were used as inocula to establish v.a. mycorrhizal development in seedlings of Allium cepa (onion) and A. porrum (leek).

2.1. Soils

Soils from various locations at the Botanical Gardens of the University of Canterbury were examined for the abundance of Endogone spores. Soil which had a low Endogone spore count (between 1 - 4 spores per g. soil) and which had a low phosphorus content, estimated by the Truog and Bray methods to be 40 mg. P per 1000 g. soil (Truog) and 15 mg. P per 1000 g. soil (Bray), was used in this study.

Soil to be sterilised was placed in quart size glass jars and covered with the bottom halves of 9 cm. petri dishes. These were then autoclaved at 121° C for 15 minutes.

Soil not to be sterilised was similarly placed in quart size glass jars.

2.2. Inocula

Spores of Endogone mosseae were isolated by the adhesion-floatation method from the roots of A. cepa which had been inoculated at the seedling stage and established in autoclaved soil.

2.3. Test plants

A. cepa and A. porrum seeds were surface sterilised in 2% sodium hypochlorite for 5 minutes and washed thoroughly five times with sterile water. They were then germinated on filter paper in 20 cm. petri dishes at 20°C in growth rooms illuminated for 16 hours daily. These seedlings were watered lightly twice weekly with sterile water. After two weeks, they were examined and seedlings of uniform size were selected for treatment.

2.4. Introduction of Endogone inocula to plants

Inoculation of plants was conducted under a hood which was thoroughly swabbed with 70% alcohol. A 15 cm. depression was made in the sterilised soil in the centre of a glass jar, into which Endogone spores were washed with sterile water. A two week old seedling was then placed into this depression, thus ensuring that the emergent roots of the seedling would be adjacent to the Endogone spores. After covering the roots and stabilising the seedling, a preserving seal was placed on the glass jar, with the seedling emerging through an opening punched in the centre of the seal. The seal also had a smaller opening carrying a rubber bung through which watering was

affected. The whole jar was then covered up by aluminium foil up to the seedling. As the seedling grew, it pushed its way through the aluminium foil and thus was not restricted in its growth. The aluminium foil thus not only protected the sterilised media from contamination but also kept light away from the roots. In the control treatments, two week old seedlings were treated with the leachings left over from the separating funnel. This ensured that all plants received the same microbial treatment but that test plants were given Endogone spores in addition.

The plants were placed in growth rooms at 20°C, illuminated for 16 hours daily and watered once weekly. At 3, 5, 8, 12, 16, 20 and 24 week intervals, records were made of the dry weight of the root and shoot systems, height of plant, number of roots per plant and the percentage root colonised by v. a. mycorrhiza.

2.5. Determination of % root colonisation by Endogone

To assess mycorrhizal development at each harvest, roots of the plant were carefully cleaned to remove soil, cut into 1 cm. segments and fixed in formalin-acetic acid-alcohol for 12 hours. The root segments were then cleared in 10% KOH at 90°C for one hour. They were then transferred to petri dishes and stained with 0.05% trypan blue in lactophenol. Excess stain was washed off with lactophenol. The stained segments were examined at 100 X magnification to determine the extent of mycorrhizal development. Stained root segments were then mounted on slides and photographed.

V. a. mycorrhizal development was also estimated by

determining the dry weight of mycorrhizal roots per plant. This was achieved by multiplying the dry weight of the root system by the determined percentage of roots colonised by Endogone.

2.6. Field trials

A 3:1 unsterilised soil-sand mixture of low phosphate content (less than 40 mg. P per 1000 g. soil, as estimated by the Troug method) and low Endogone spore count (2-5 spores per g. soil) was used. Two week old seedlings, selected for uniformity, were placed 15 cm. apart in rows placed 90 cm. apart, on a field plot near the glasshouses at the Botanical Gardens of the University of Canterbury. Test seedlings were treated with Endogone spores while control seedlings received left over leachings.

Rigorous weed control was employed during the period of the experiment. The plants were watered twice weekly and harvested at 3, 5, 8, 12, 16, 20 and 24 week intervals. Records were made of percentage root colonisation by Endogone and of dry weight of shoot and root systems and bulbs.

3. V. A. MYCORRHIZAE IN PATHOGENESIS

Most of the research done to date pertaining to the beneficial effect that Endogone has on plants have been concerned with an improved nutritional status on the part of the plant. This study was set up to determine whether this beneficial effect may be extended to cover increasing the host plant's resistance to disease. Thus, the

interaction between v. a. mycorrhizal fungi and certain soil borne fungal pathogens was studied under controlled as well as field conditions.

3.1. Pathogens under study

Cultures of the fungal pathogens Fusarium oxysporum var. cepa and Pyrenochaeta terrestris were obtained from the Plant Diseases Division, D.S.I.R., Auckland and subcultured on potato dextrose agar slants in McCartney bottles at 25°C.

3.2. Inoculation of onions with pathogens

Both control and test onion plants, prepared as previously described were inoculated with the pathogens. The plants, growing in the glass jars in the growth rooms were again placed in a hood which had been thoroughly swabbed with 70% alcohol. The aluminium foil covering the whole jar was carefully opened up so as not to damage the plants.

When required, the agar slants containing the pathogens were three-quarter filled with sterilised water and lightly agitated with a sterilised loop to free the fungal hyphae from the agar. The water and loosened fungal hyphae was taken up by a sterile hypodermic syringe fitted with a 50 mm. needle and this was injected deep into the sterilised soil immediately adjacent to the roots of an onion plant. The aluminium foil was then carefully replaced and the plant returned to the growth room. The plants were later examined for the development of disease symptoms.

The same technique was adopted for field trials, but

to prevent cross infections in the field, the different pathogens were applied at least 6 meters apart.

The dry weight of the shoot and root systems and bulbs and the percentage root colonisation by Endogone and dry weight of mycorrhizal roots of resistant plants were recorded.

4. THE IMPORTANCE OF PHOSPHATE NUTRITION ON PLANT GROWTH AND DISEASE RESISTANCE

A decrease in the incidence of disease symptoms of F. oxysporum v. cepa and P. terrestris may have been due to an improved nutritional status of mycorrhizal plants, as a result of an increased phosphate uptake. Direct application of extra phosphate may just have achieved similar levels of resistance. A study was conducted to determine whether any increased resistance may have been merely due to an increased phosphate uptake and content of the plant or that the resistance may have been attributed to some other mechanism associated with v. a. mycorrhizal development.

4.1. Test plants

Two week old onion seedlings were planted in sterilised soil as previously described, except that 15 cm. pots were used as the containers instead. These pots had been sterilised by autoclaving. Half of these seedlings were inoculated with Endogone spores while the other half received left over leachings. The tops of these pots were then closed over with aluminium foil.

4.2. Media

The plants were given $CaHPO_4.2H_2O$ as the main source of phosphate. Different treatments received 0.1 g. $CaHPO_4.2H_2O$/kg. soil, 0.5 g. $CaHPO_4.2H_2O$/kg. soil, 1.0 g. $CaHPO_4.2H_2O$/kg. soil and 2.0 g. $CaHPO_4.2H_2O$/kg. soil. The plants were watered twice weekly with a nutrient solution (Hewitt, 1966) made up of the following: 0.002M $Ca(NO_3)_2$, 0.004M KNO_3, 0.002M $MgSO_4$ and 0.004M $(NH_4)_2.SO_4$. This was made up in de-ionised water.

4.3. Inoculation of onions with pathogens

The plants were placed in a glass house for 12 weeks at an average temperature of 15°C, with 16 hours of illuminition daily. The pathogens were introduced to the plants after 10 weeks, in a similar manner as described previously. Records were made of dry weight of root and shoot (including bulb) systems, percentage root colonisation by Endogone and weight of phosphorus per 100 mg. dry matter.

5. CULTURING V.A. MYCORRHIZA IN A. CEPA IN AGAR MEDIA

A study was conducted to investigate the possibility of establishing v.a. mycorrhizal development in A. cepa in agar media which varied in the source of phosphate. Ungerminated, surface sterilised spores of Endogone were used.

5.1. Inocula

Endogone spores, obtained as described previously, were surface sterilised in 2% (w/v) Chloramine T containing 200 mg. streptomycin/litre and a trace of detergent (Mosse, 1961). The spores were transferred by capillary pipettes to

watch glasses containing the sterilant and then rinsed in 5 changes of sterile water.

5.2. Media

All treatments contained, in addition to that tabled below, the following: Difco Bacto Agar 15 g. ; KNO_3 0.5 g. ; $MgSO_4 . 7H_2O$ 0.2 g. ; $CaCl_2$ 0.2 g. per litre distilled water. All media were sterilised at 121° C for 15 minutes.

5.2.1. Composition of media (g./l.)

Medium		mg. P/l.
1	0.1 $FeCl_3$ (35 mg. Fe)	0
2	0.1 $FeCl_3$ + 0.55 $CaHPO_4 . 2H_2O$	100
3	0.1 $FeCl_3$ + 0.12 Inositol	0
4	0.1 $FeCl_3$ + 0.63 Ca phytate	100
5	0.1 $FeCl_3$ + 0.63 Na phytate	100
6	Fe K EDTA (36 mg. Fe)	0
7	Fe K EDTA + 0.55 $CaHPO_4 . 2H_2O$	100
8	Fe K EDTA + 0.12 Inositol	0
9	Fe K EDTA + 0.63 Ca phytate	100
10	Fe K EDTA + 0.63 Na phytate	100

5.3. Test plants

Onion seeds were surface sterilised and germinated as previously described. Two week old seedlings were placed on agar slants in boiling tubes and placed in a growth room at 20° C with 16 hour daily illumination for 10 weeks. Sterile water was added to each plant.

5.4. Measurements

The plants were first tested for contamination (Mosse, 1956) by adding some supernatant liquid from each

boiling tube to sterile nutrient broth (Oxoid) in which Endogone does not grow. Liquid from monoxenically inoculated, uncontaminated seedlings left the broth clear. The tubes were then immersed in boiling water to melt the agar and the plants were removed, rinsed in warm water and dried between filter paper. Records were made of dry weight of shoot and root systems, percentage root colonisation by Endogone and percentage P in plant.

6. GERMINATION OF ENDOGONE SPORES

A study was conducted to compose a medium that would bring about the germination of Endogone spores most readily under laboratory conditions.

6.1. Inocula

Endogone spores were obtained from two sources, namely from soil in which spores were maintained with A. cepa (spore source 200) and with A. porrum (spore source 100). The spores, isolated and stored as described previously, were surface sterilised in streptomycin/ chloramine T and a trace of detergent (Mosse, 1962) and rinsed in sterile water. Twenty-five spores were placed 20 mm. apart in each petri dish of test medium and incubated in the dark at 20°C. Two incubators were used, one metal and the other wooden.

6.2. Media

All test media were prepared using sterile water and the pH adjusted to 7.0 before being autoclaved at 121°C for 15 minutes.

6.2.1. Composition of media (per 1. sterile water)

Medium

1. 10 g. Difco Bacto Agar, 0140-01 (water agar)
2. Water agar + 0.55 g. $CaHPO_4.2H_2O$
3. Water agar + 0.63 g. Ca phytate
4. Water agar + 0.12 g. Inositol
5. Water agar + 0.5 mg. nicotinic acid
6. Water agar + 0.1 mg. thiamine HCl
7. Medium 2 + 0.5 mg. nicotinic acid
8. Medium 3 + 0.5 mg. nicotinic acid
9. Medium 4 + 0.5 mg. nicotinic acid
10. Medium 2 + 0.1 mg. thiamine HCl
11. Medium 3 + 0.1 mg. thiamine HCl
12. Medium 4 + 0.1 mg. thiamine HCl
13. Medium 2 + 0.5 mg. nicotinic acid + 0.1 mg. thiamine HCl
14. Medium 3 + 0.5 mg. nicotinic acid + 0.1 mg. thiamine HCl
15. Medium 4 + 0.5 mg. nicotinic acid + 0.1 mg. thiamine HCl
16. Water agar + 0.5 mg. nicotinic acid + 0.1 mg. thiamine HCl

6.3. Measurements

Records were made of the number of Endogone spores germinated every 2 days, up to 14 days.

7. ULTRASTRUCTURAL STUDIES OF V.A. MYCORRHIZA IN THE ROOTS OF ALLIUM CEPA

A scanning electron microscope (SEM) was used to

study the morphological aspects of v.a. mycorrhizae in the onion at the ultrastructural level, employing a modified version of the technique developed by Kinden and Brown (1974).

7.1. Test plants

The roots of Endogone treated, twenty week old, onion plants grown in sterilised soil at 20°C with 16 hours illumination per day were carefully washed to remove soil particles and cut into 0.5-1.0 cm. sections.

7.2. Preparation of material

The root sections were fixed in a buffered 5% glutaraldehyde solution at room temperature and left overnight. The buffer used was 0.1M KH_2PO_4 - K_2HPO_4, at pH 6.8 - 7.2. After 5 buffer rinses and an overnight wash to remove excess fixative, the specimens were left to post - fix overnight in buffered 2% OsO_4. Following another 5 rinses in buffer, the samples were cut longitudinally with a sharp razor blade. This exposed the maximum interior for the removal of host cytoplasm.

The specimens were treated with 1.0% periodic acid for 3 minutes, rinsed in distilled water 6 times over a 10 minute period andthen placed in 4% KOH at 45°C for 30 minutes. The samples were then placed in 1.0% acetic acid for 5 minutes, rinsed with distilled water 10 times over a 1 hour period and placed overnight in buffered 2% OsO_4. After 6 buffer rinses to remove excess OsO_4, the specimens were dehydrated in the following graded ethanol series for the duration indicated:

20% ethanol	1 hour
40% ethanol	10 hours
60% ethanol	1 hour
80% ethanol	1 hour
95% ethanol	1 hour
100% ethanol	1 hour - overnight

The ethanol was replaced with amyl acetate in the following ethanol - amyl acetate series:

30% amyl acetate (in ethanol)	1 hour
50% amyl acetate (in ethanol)	5 hours
70% amyl acetate (in ethanol)	1 hour
90% amyl acetate (in ethanol)	10 hours
100% amyl acetate (in ethanol)	1 hour - overnight

The specimens were then critical point dried with carbon dioxide, mounted on double sided cellotape on stubs and coated with gold in a diode sputterer. These were examined with a Cambridge Stereoscan 600 scanning electron microscope operating at 25 Kev.

Endogone treated plants which were resistant to F. oxysporum v. cepa and P. terrestris were similarly treated and examined.

CHAPTER IV

ISOLATION OF *ENDOGONE* SPORES

1. RESULTS

The method employed in the present study to isolate *Endogone* spores was easily set up and proved very simple but efficient in its operation. Occassionally, fragments of organic matter and soil particles were scattered among the *Endogone* spores, but not seriously enough to interfere with the recognition and counting of the spores when the filter papers were viewed under a binocular microscope. The spores ranged in size from 50 to 250 µm in diameter, varying little with soil depth and season of the year. The colour of the spores varied from pale yellow to dark brown, although yellow spores predominated and were between 170 to 220 µm in diameter.

Endogone spore populations varied according to depth of sampling as shown in Table 1. The spores were concentrated in the 15 - 25 cm. region, declining rapidly with further increase in depth in the soils examined.

The mean number of spores per g. of dried soil also varied at different times of the year and was influenced by the time of planting of the host onions. In soils in which onions were planted in March, 1974, that is in autumn planted soils, spore numbers were high in the following July (about 6 - 40 spores/g. soil), reaching a maximum in October, 1974 (about 9 - 48 spores/g. soil), but falling in

Table 1. Isolation of Endogone spores.

Soil type	Depth of Sample (cm)	Mean number of spores per gram dried soil(5 replicates)						
		Time of sampling						
		July 1974	October 1974		January 1975		March 1975	
		Planting season of host plants						
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Loam	5-15	23	10	30	15	18	36	13
	15-25	36	29	45	33	15	50	25
	25-40	10	7	13	10	3	25	8
Loam	5-15	21	22	35	20	7	37	15
	15-25	40	30	48	38	13	45	18
	25-40	16	15	10	13	4	22	9
Sandy loam	5-15	14	8	22	10	7	18	12
	15-25	26	24	35	30	13	33	20
	25-40	6	11	9	7	3	14	8
Sandy loam	5-15	16	10	25	12	10	27	20
	15-25	23	19	30	29	15	37	19
	25-40	9	6	12	10	3	20	6

January, 1975 (about 3 - 18 spores/g. soil). Endogone spore populations recorded in the 15 - 25 cm. region in October, 1974 were significantly greater than those recorded in January, 1975. Endogone spore numbers in spring planted (September, 1974) soils were low in the following October (6 - 30 spores/g. soil), increasing slightly in January, 1975 (7 - 38 spores/g. soil) and climbing to a maximum in March 1975 (14 - 50 spores/g. soil). Endogone spore numbers found in the 15 - 25 cm. region in October, 1974 were significantly lower than those recorded in March, 1975.

2. DISCUSSION

The floatation - adhesion technique proved efficient in recovering Endogone spores quantitatively. Most other workers have recovered smaller spore populations than those recorded here. Gerdemann and Nicolson (1963), Hayman (1970), Mason (1964), Nicolson (1967) and Schenck and Hinson (1971) reported spore populations ranging from 0.05 - 4.0 spores/g. soil. Mosse and Bowen (1968) found 3 - 33 spores/g. soil. Sutton and Barron (1972) recovered 20 - 86 spores/g. soil, using the floatation - adhesion method of spore isolation. In this study, spore populations ranging from 0 - 50 spores per g. soil were recovered. This is lower than that reported by Sutton amd Barron, but considerably greater than those reported by the other mentioned workers.

It is possible that the greater spore population recovery by Sutton and Barron (1972) and in the present work, is due to the larger size range of spores being recovered. With the wet - sieving method, spores passing

through the smallest sized sieve, the 100μ sieve, would have been discarded and thus lost. Sutton and Barron (1972) found that 20 - 70% of the spores recovered were less than 100μ in diameter.

Endogone spores were found in the regions of soil closer to the upper regions of the roots, especially at depths between 15 - 25 cm. It was interesting to note that, of the regions of the roots that show mycorrhizal development, the upper regions were most heavily infected, as indicated by the yellowing of the infected upper regions. This correlation between numbers of spores and extent of mycorrhizal development has also been reported by Hayman (1970) and Daft and Nicolson (1972). Conversely, Redhead (1971) failed to find spore numbers to be related to root colonisation in Nigerian soils and Mosse (1973) did not recover any spores from Nigerian rain forest soils and in some New Zealand bush soils amongst plant species which nevertheless demonstrated extensive mycorrhizal development in the root cortex.

Spore populations recorded in this study differed appreciably according to when the host plants were planted. In soils where onions were planted in early spring, spore numbers increased slightly in the following summer and reached a maximum in autumn, but falling in winter. The maximum spore numbers found in autumn were recorded just before the onions were harvested. Sutton and Barron (1972) reported similar findings, working on maize, strawberry, tomato and wheat in four agricultural soils. They found

that spore populations generally increased in late summer and autumn but concluded that seasonal changes in Endogone spore numbers were generally minimal. Similar spore population figures were also reported by Mejstrik (1972), working on a Molinietum coeruleae association. He found that most spores were recorded in autumn and least in spring.

In soils where onions were planted in early autumn, spore numbers increased in winter and reached a maximum in the following spring. The spore populations then diminished in the following summer and autumn. Again it was observed that the maximum recorded occured just before the onions were harvested. A similar peak in spore numbers in winter was reported by Mason (1964), working on strawberry. Conversely, working in Great Britain, Mason (1964) and Hayman (1970) found increased spore populations in summer, which declined in autumn. Redhead (1971) similarly found seasonal spore number fluctuations in Nigeria.

Fluctuations in spore numbers seem to be related to the stage of development of the host plant. When the seedlings were first planted, either in autumn (March, 1974) or in spring (September, 1974), spore numbers were low, but just before the onions were harvested, spore numbers reached a maximum. Mosse and Bowen (1968) suggested that spore production occurs chiefly during intermittent root growth and Mosse (1959) and Hayman (1970) concluded that spore populations increase after a period of maximum root growth.

When the onions were first planted, root growth was continuous, as shown in figure 7 and spore numbers were found to be low, as shown in Table 1. But just before these host plants were harvested, root growth had reached its maximum and starting to level off, with spore numbers being correspondingly high. These findings are in agreement with those reported by Hayman (1970), Mosse (1959) and Mosse and Bowen (1968). These workers concluded that spore production was greatest when the roots of the associated host plants had reached maximum growth. The seasonal decline in spore numbers may be caused by stimulation of spore germination in the colonisation of available root tissue (Dowding,1959; Gerdemann, 1968; Mosse and Bowen, 1968) or by death of spores through ingestion by soil fauna or destruction by parasites.

The sudden increase in spore numbers in the winter of 1974 in autumn planted soils may be accounted for by root growth being temporarily arrested by cold, thereby reducing the availability of roots for Endogone colonisation. Spore germination is probably reduced, causing an increase in spore numbers recovered. In addition, the cold may have created a situation of intermittent root growth and the high spore numbers recorded would be in agreement with Mosse and Bowen's (1968) findings.

These fluctions in spore numbers indicate that great care should be taken when quantitative sampling of spores is carried out at various times of the year. It is important to realise that Endogone spore populations are

influenced not only by seasonal changes, but also by the stage of development of the host plants.

Figure 1. Apparatus used in the isolation of Endogone spores

CHAPTER V

EFFECT OF *ENDOGONE* ON *ALLIUM* SPECIES

1. RESULTS

1.1 Effect of *Endogone* on *Allium cepa* growth

1.1.1. Percentage of root colonisation by *Endogone*

Root colonisation occurred as early as the third week of growth of onion plants after inoculation with *Endogone*. The rate of colonisation was then very rapid until the 12th week, after which it began to level off. This occurred under both controlled and field conditions. Up to 75% root colonisation occurred in the former and 80% in the latter.

Under controlled conditions, the rate of colonisation by *Endogone* was significantly greater in plants grown in sterilised soil than in unsterilised soil (Table 2). All control plants (i. e. not treated with *Endogone*) did not become mycorrhizal, except in one instance, when 3% became mycorrhizal at the 16th week harvest.

Under field conditions, colonisation was recorded only after 5 weeks, but the rate increased rapidly. Control plants showed a low incidence of colonisation, not exceeding 6%.

Figures 9, 10 and 11 show photographs of the mycorrhizal fungus and the hyphae are seen to be typically aseptate, densely staining and exhibiting characteristic wide - angled dichotomous branching.

Arbuscules, shown in figures 10a and 10b, were found in young and mature plants, that is, 3 - 20 week old plants but were uncommon in older plants, that is, 24 week old plants. Vesicles, shown in figure 11, were not present in young plants, but were found in mature and older plants.

1.1.2. Effect of Endogone on dry weight of onion root system. Plants grown in the presence of Endogone showed significantly greater root growth than control plants in both controlled and field conditions (Table 3).

Under controlled conditions, the differences between control and test plants were not apparent until the 8th week harvest (Figure 3). By the 20th week, the dry weight of the root system of test plants grown in sterilised soil (1.394 g.) was almost double that of the controls (0.754 g.). Among the test plants, those grown in sterilised soil had a significantly higher root production than those in unsterilised soil (Table 3). Control plants grown in sterilised and unsterilised soil exhibited very similar root growth rates.

Plants grown in the field generally showed greater root growth rates than those grown in the growth rooms, as shown in figure 3.

1.1.3. Dry weight of mycorrhizal roots. Another means of estimating the extent of v.a. mycorrhizal development is by multiplying the dry weight of the root system/plant by the percentage of root colonisation by Endogone/plant (Sutton and Barron, 1972).

Under controlled conditions, significantly greater dry weight of mycorrhizal roots were recorded in plants grown in sterilised soil than in unsterilised soil (Table 4). Control plants showed a significantly lower recording of dry weight of mycorrhizal roots than test plants under both controlled and field conditions, with those grown in the field resulting in a higher recording of dry weight of mycorrhizal roots than those placed in the growth rooms.

1.1.4. Effect of Endogone on the number of roots produced per onion plant. This was only recorded of the onions grown under controlled conditions, as shown in figure 5. All treatments followed similar growth trends. Plants treated with Endogone were shown to produce a significantly greater number of roots than control plants, with lesser root growth in unsterilised soil than in sterilised soil (Table 5).

1.1.5. Effect of Endogone on the dry weight of onion shoot system. The differences in the dry weight of the shoot system between test and control plants were not apparent until the 12th week harvest, as shown in figure 6. Again, the presence of Endogone resulted in significantly heavier shoot systems (Table 5). At the 20th week harvest, the mean dry weight of the shoot system of test plants grown in sterilised soil (2.373 g.) almost doubled those of control plants (1.335 g.). In control plants, the rate of shoot growth was similar in sterilised and unsterilised soils.

1.1.6. Effect of Endogone on the height of onion plants. This was only recorded from onions grown under controlled conditions, as shown in figure 7. Control

plants grown in sterilised and unsterilised soil and test plants grown in unsterilised soil showed very similar growth patterns. Conversely, test plants grown in sterilised soil exhibited significantly greater weight.

1.1.7. Effect of Endogone on the dry weight of onion bulb. This was recorded only from plants grown under field conditions, as shown in figure 8. After the 12th week harvest, test plants were by far significantly greater in bulb weight than control plants (Table 6).

Figure 2. Onion root colonisation by Endogone.

● With Endogone; o Without Endogone;

——Sterilised soil; ·—·—Unsterilised soil;

C=Controlled Conditions; F=Field Conditions;

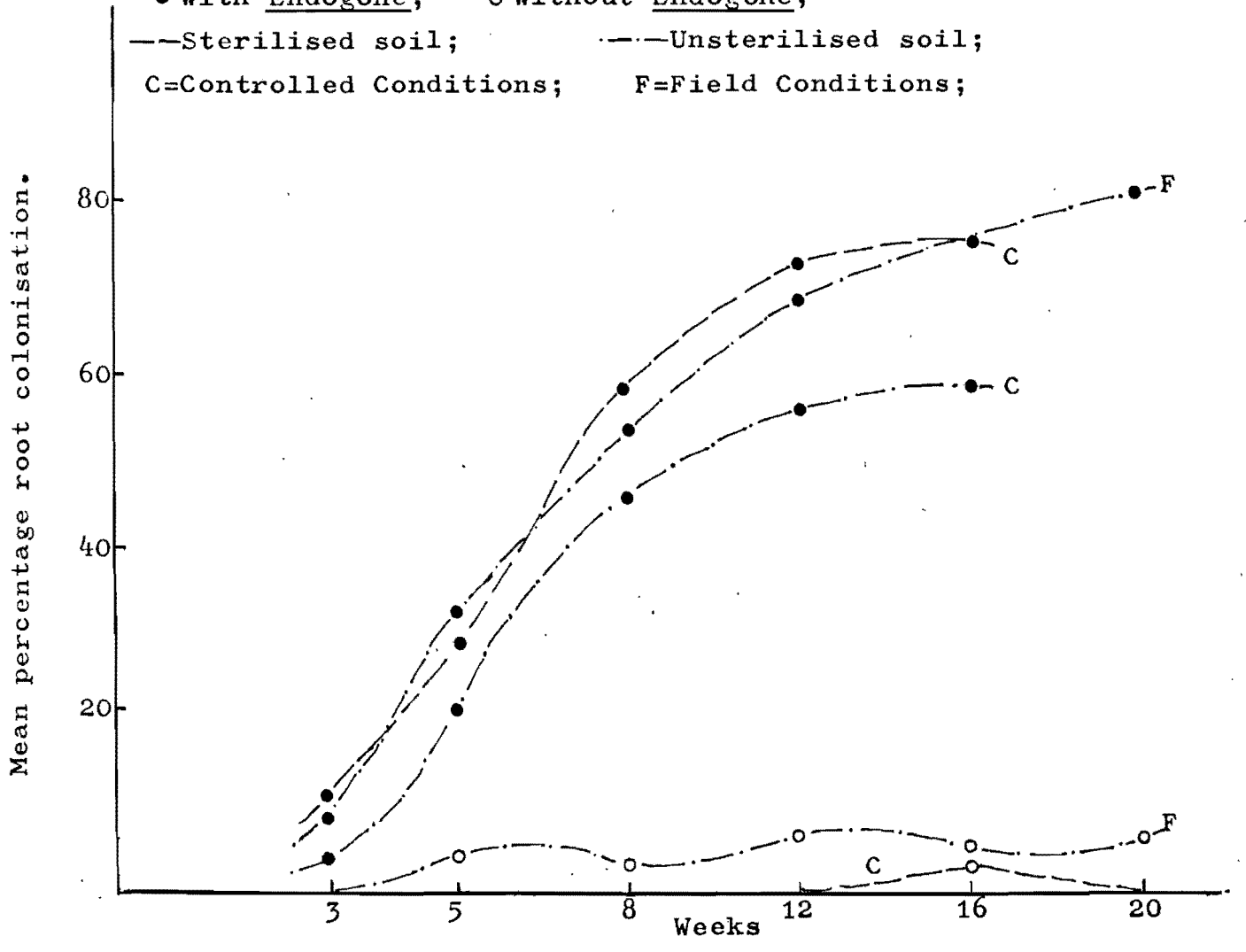


Table 2. Comparison of onion root colonisation by Endogone under Controlled and Field Conditions (5 replicates). End. = Endogone

Weeks after trans-planting	Soil treatment	% root colonisation per plant (back-transformed) Controlled +End.	Controlled -End.	Field +End.	Field -End.	Analysis of Variance (5%) S...Significant difference I...Insignificant difference
3	Sterilised	3.000^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.000^{B}	1.000^{D}	1.000^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
5	Sterilised	12.000^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: I;
	Unsterilised	5.000^{B}	1.000^{D}	1.451^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
8	Sterilised	29.005^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	22.002^{B}	1.000^{D}	33.001^{F}	5.000^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
12	Sterilised	59.007^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	45.995^{B}	1.000^{D}	53.995^{F}	4.000^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
16	Sterilised	73.005^{A}	4.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	57.005^{B}	1.000^{D}	68.992^{F}	6.997^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
20	Sterilised	76.000^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	59.007^{B}	1.000^{D}	76.190^{F}	5.999^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
24	Unsterilised			80.997^{F}	7.003^{H}	F Vs H: S;

Figure 3. Dry weight of root system of onions.

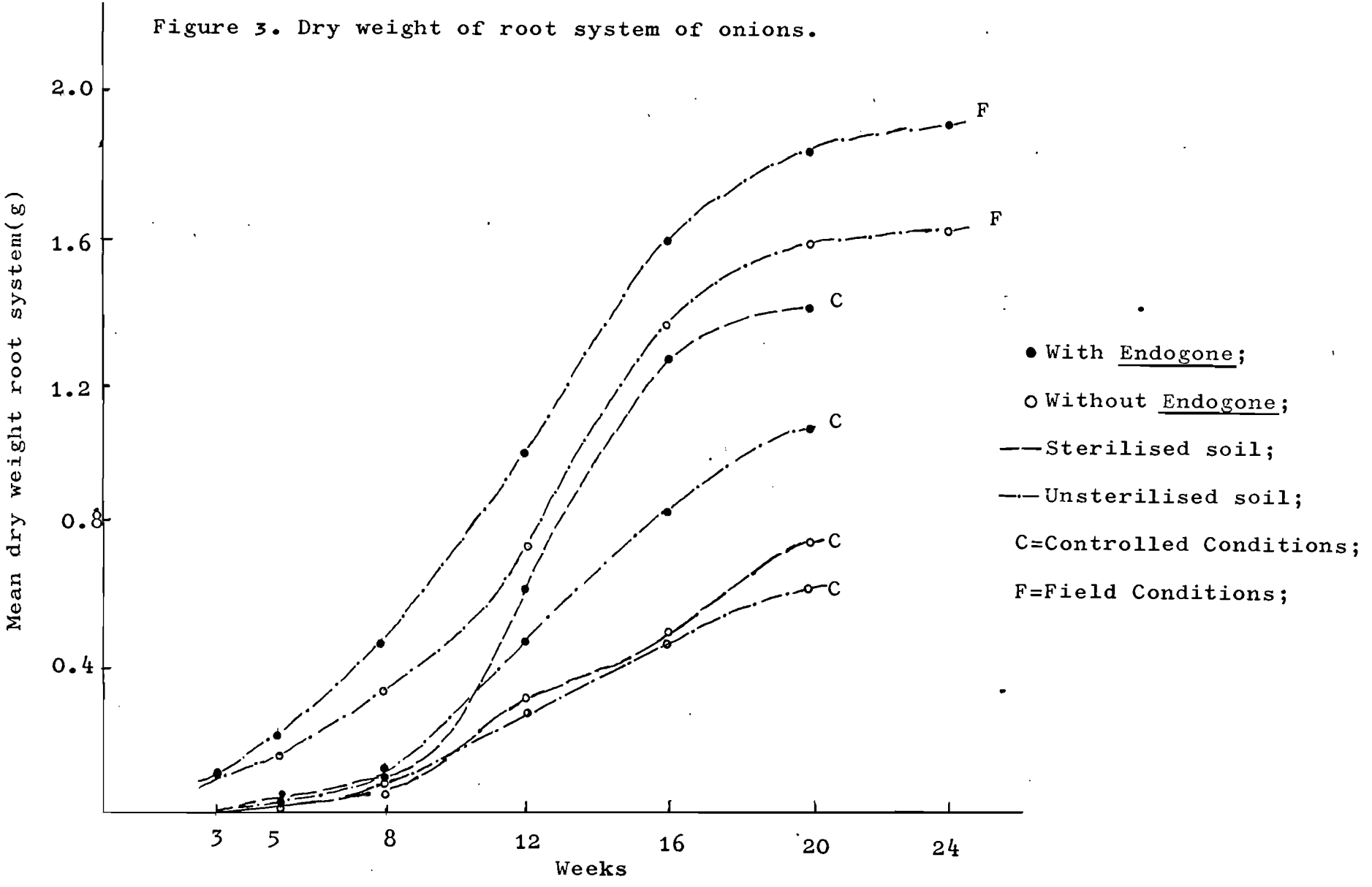


Table 3. Comparison of dry weight onion root system under Controlled and Field Conditions. (5 replicates). End. = Endogone

Weeks after transplanting	Soil treatment	Dry weight root system per plant (g.) Controlled +End.	Controlled -End.	Field +End.	Field -End.	Analysis of Variance(5%) S = Significant difference I = Insignificant difference
3	Sterilised	0.010^{A}	0.010^{C}			A Vs B; I; A Vs C: I; B Vs D: I;
	Unsterilised	0.010^{B}	0.010^{D}	0.100^{F}	0.092^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
5	Sterilised	0.028^{A}	0.018^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	0.025^{B}	0.020^{D}	0.224^{F}	0.165^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
8	Sterilised	0.110^{A}	0.063^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	0.120^{B}	0.007^{D}	0.483^{F}	0.350^{H}	B Vs F: I; D Vs H: S; F Vs H: I;
12	Sterilised	0.620^{A}	0.330^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	0.487^{B}	0.286^{D}	1.051^{F}	0.748^{H}	B Vs F: S; D Vs H: S; F Vs H: S;
16	Sterilised	1.260^{A}	0.500^{C}			A Vs B: S; A Vs C: S; B Vs D; S;
	Unsterilised	0.844^{B}	0.474^{D}	1.614^{F}	1.367^{H}	B Vs F: S; D Vs H: S; F Vs H: S
20	Sterilised	1.391^{A}	0.754^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	1.066^{B}	0.623^{D}	1.875^{F}	1.584^{H}	B Vs F: S; D Vs H: S; F Vs H: S;
24	Unsterilised			1.926^{F}	1.625^{H}	F Vs H: S;

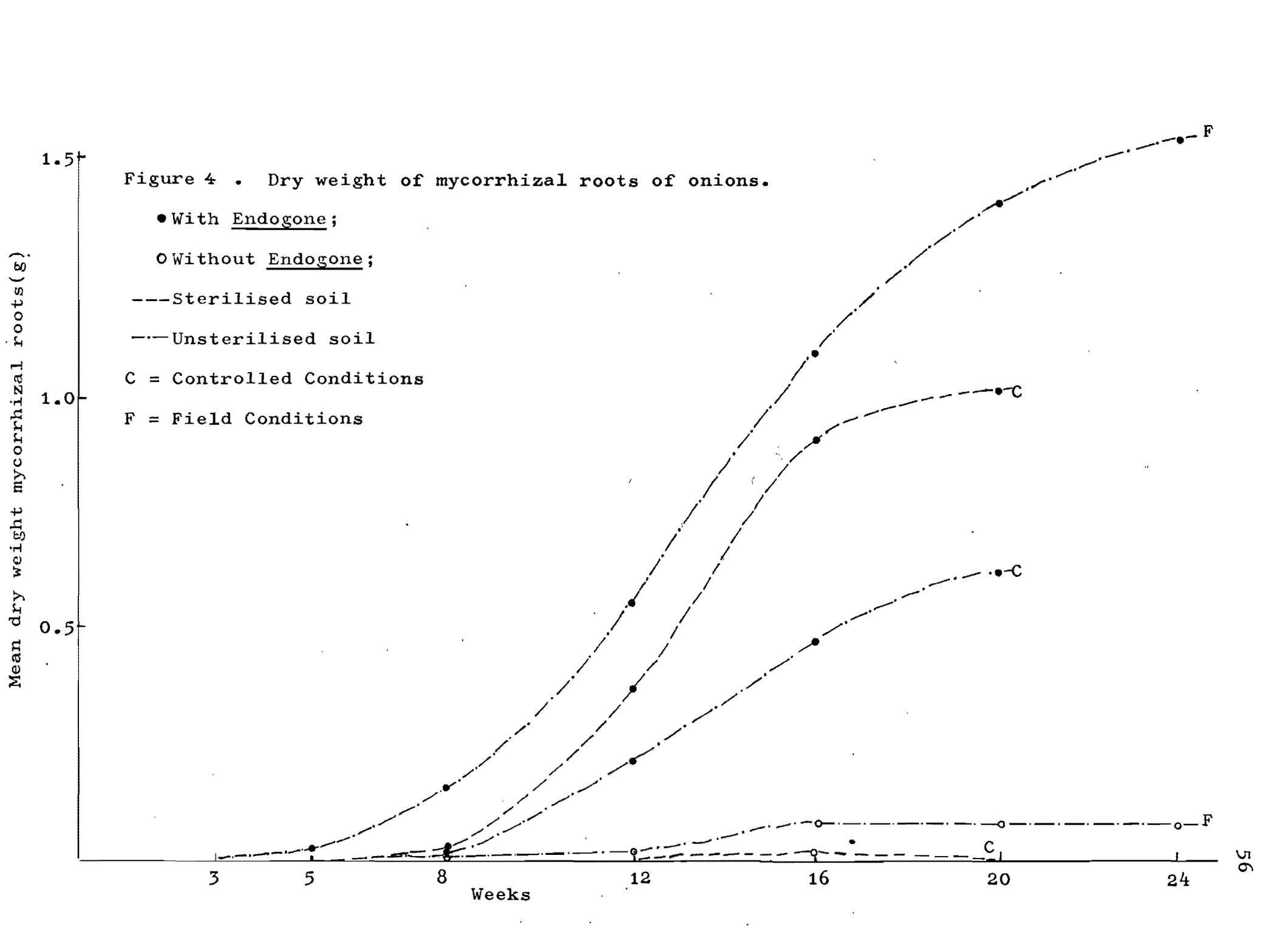
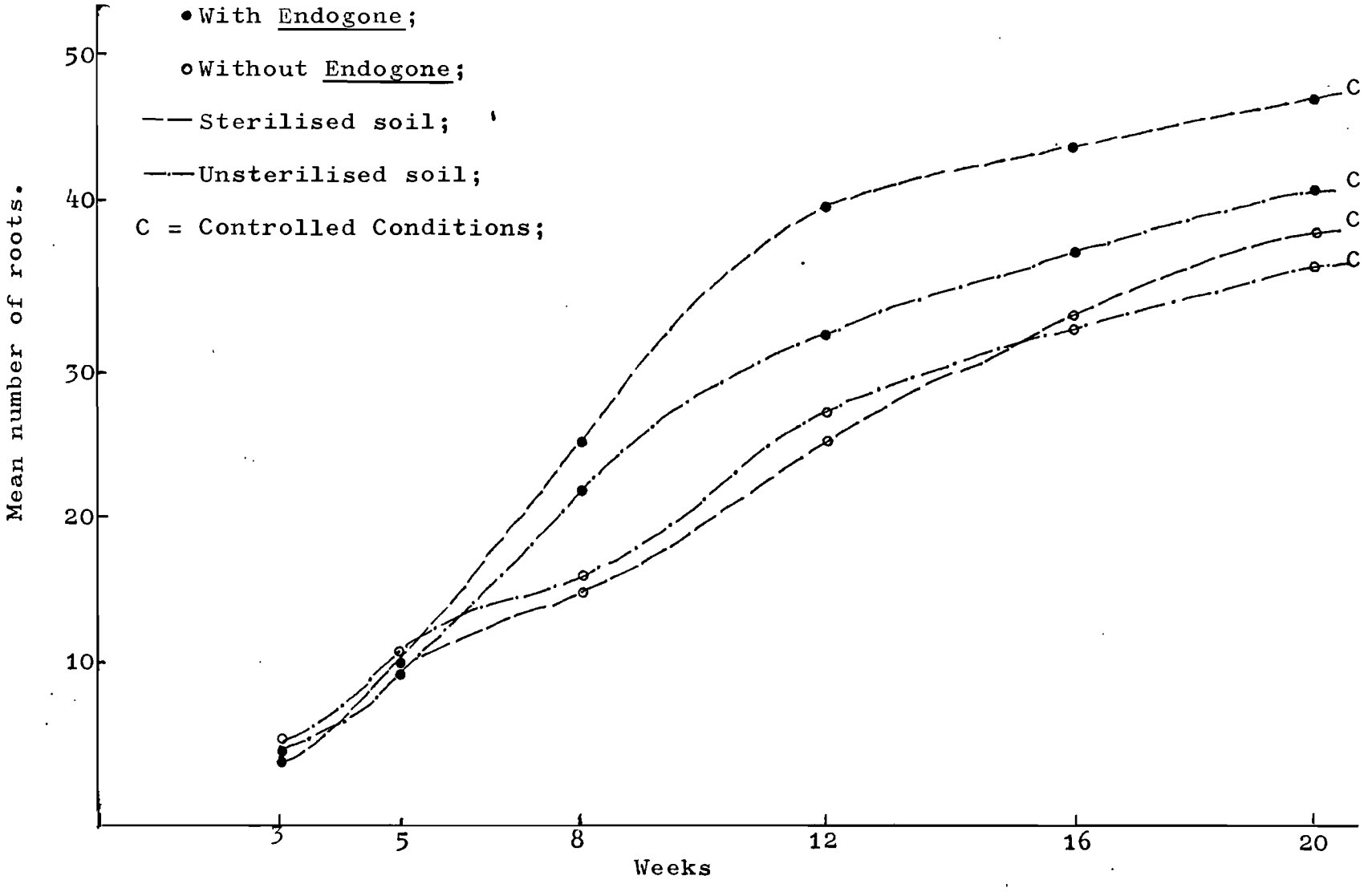


Figure 4 . Dry weight of mycorrhizal roots of onions.
● With Endogone;
○ Without Endogone;
---Sterilised soil
—·—Unsterilised soil
C = Controlled Conditions
F = Field Conditions
Mean dry weight mycorrhizal roots(g)
1.5
1.0
0.5
3
5
8
12
16
20
24
Weeks
F
C
C
F
C

Table 4. Comparison of dry weight onion mycorrhizal roots under Controlled and Field Conditions. (5 replicates). End. = Endogone

Weeks after trans-planting	Soil treatment	Dry weight mycorrhizal roots per plant (back-transformed) Controlled +End.	Controlled -End.	Field +End.	Field -End.	Analysis of Variance (5%) S...Significant difference I...Insignificant difference
3	Sterilised	1.018^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.000^{B}	1.000^{D}	1.000^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
5	Sterilised	1.003^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.001^{B}	1.000^{D}	1.085^{F}	1.004^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
8	Sterilised	1.030^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.026^{B}	1.000^{D}	1.154^{F}	1.014^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
12	Sterilised	1.359^{A}	1.000^{C}			A Vs B: I; A Vs C: S; B Vs D: I;
	Unsterilised	1.219^{B}	1.000^{D}	1.557^{F}	1.021^{H}	B Vs F: I; D Vs H: I; F Vs H: S;
16	Sterilised	1.907^{A}	1.016^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	1.472^{B}	1.000^{D}	2.098^{F}	1.082^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
20	Sterilised	2.045^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	1.619^{B}	1.000^{D}	1.435^{F}	1.079^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
24	Unsterilised			2.617^{F}	1.098^{H}	F Vs H: S;

Figure 5. Number of roots per onion plant.



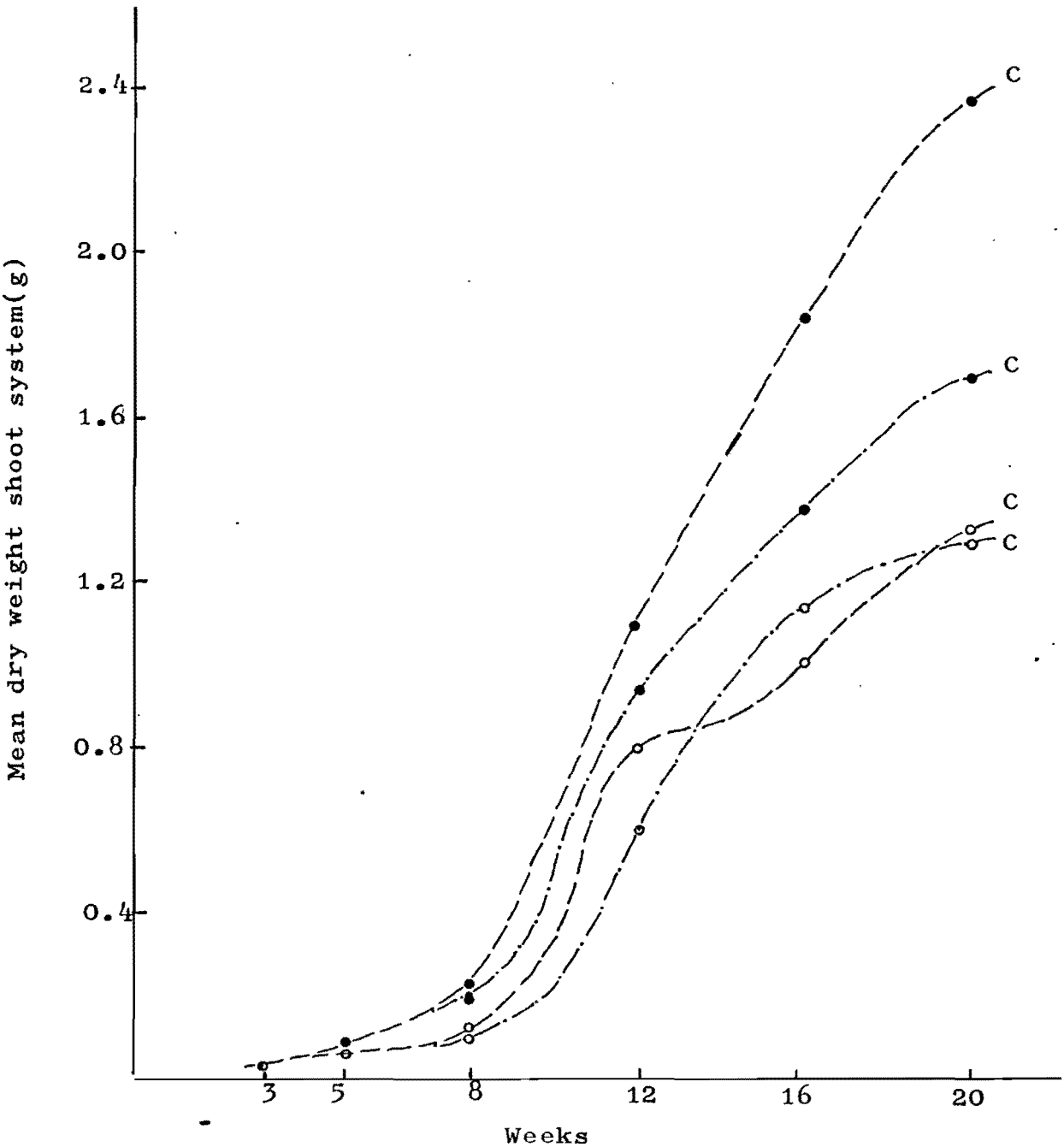


Figure 6. Dry weight of shoot system of onions.

● With Endogone;

o Without Endogone

——Sterilised soil;

—·—Unsterilised soil;

C = Controlled Conditions;

Table 5. Statiatical Analysis. Comparing Mycorrhizal and non-Mycorrhizal plants; Controlled Conditions

Ster. = Sterilised; Unster. = Unsterilised; End. = Endogone

S = Significant difference; I = Insignificant difference;

Weeks after trans-planting	Soil treatment	Mean dry weight shoot system(g.) (5 replicates) +End.	-End.	Analysis of Variance(5%)	Number of roots per plant (5 replicates) +End.	-End.	Analysis of Variance(5%)
3	Ster.	0.038^{A}	0.030^{C}	A Vs B: I; A Vs C: I;	4^{A}	5^{C}	A Vs B: I; A Vs C: I;
	Unster.	0.039^{B}	0.043^{D}	B Vs D: I; C Vs D: I;	5^{B}	5^{D}	B Vs D: I; C Vs D: I;
5	Ster.	0.081^{A}	0.071^{C}	A Vs B: I; A Vs C: I;	11^{A}	10^{C}	A Vs B: I; A Vs C: I;
	Unster.	0.077^{B}	0.062^{D}	B Vs D: I; C Vs D: I;	10^{B}	11^{D}	B Vs D: I; C Vs D: I;
8	Ster	0.243^{A}	0.130^{C}	A Vs B: I; A Vs C: I;	25^{A}	15^{C}	A Vs B: I; A Vs C: I;
	Unster.	0.214^{B}	0.100^{D}	B Vs D: I; C Vs D: I;	22^{B}	16^{D}	B Vs D: I; C Vs D: I;
12	Ster.	1.118^{A}	0.804^{C}	A Vs B: S; A Vs C: S;	40^{A}	25^{C}	A Vs B: I; A Vs C: S;
	Unster	0.955^{B}	0.602^{D}	B Vs D: S; C Vs D: I;	32^{B}	27^{D}	B Vs D: S; C Vs D: I;
16	Ster.	1.845^{A}	1.016^{C}	A Vs B: S; A Vs C: S;	44^{A}	33^{C}	A Vs B: I; A Vs C: S;
	Unster.	1.377^{B}	1.147^{D}	B Vs D: S; C Vs D: I;	37^{B}	32^{D}	B Vs D: S; C Vs D: I;
20	Ster.	2.373^{A}	1.335^{C}	A Vs B: S; A Vs C: S;	47^{A}	38^{C}	A Vs B: I; A Vs C: S;
	Unster.	1.702^{B}	1.291^{D}	B Vs D: S; C Vs D: I;	41^{B}	36^{D}	B Vs D: S; C Vs D: I;

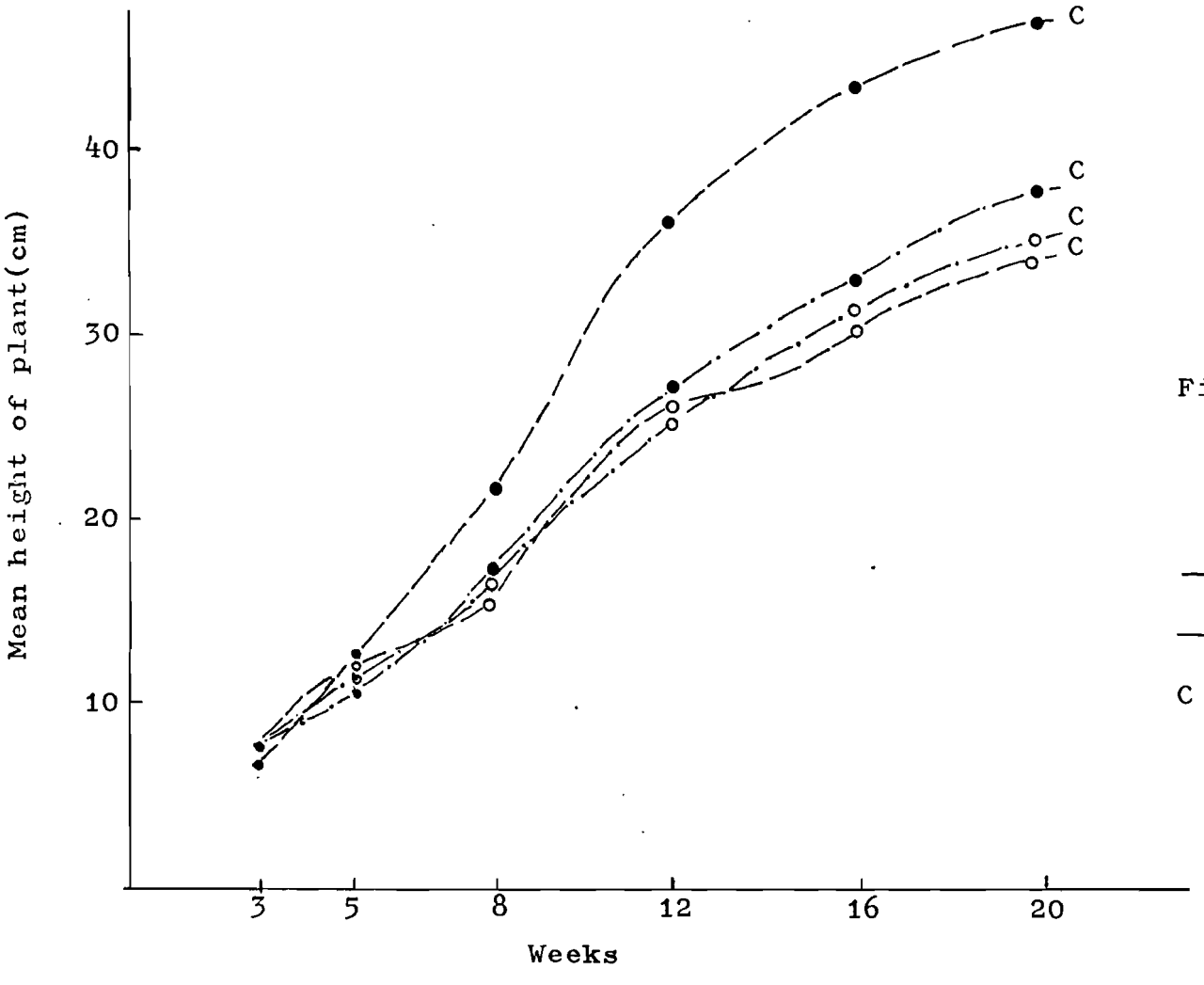


Figure 7. Height of onion plants.

● With Endogone;

o Without Endogone;

—— Sterilised soil;

—·— Unsterilised soil;

C = Controlled Conditions;

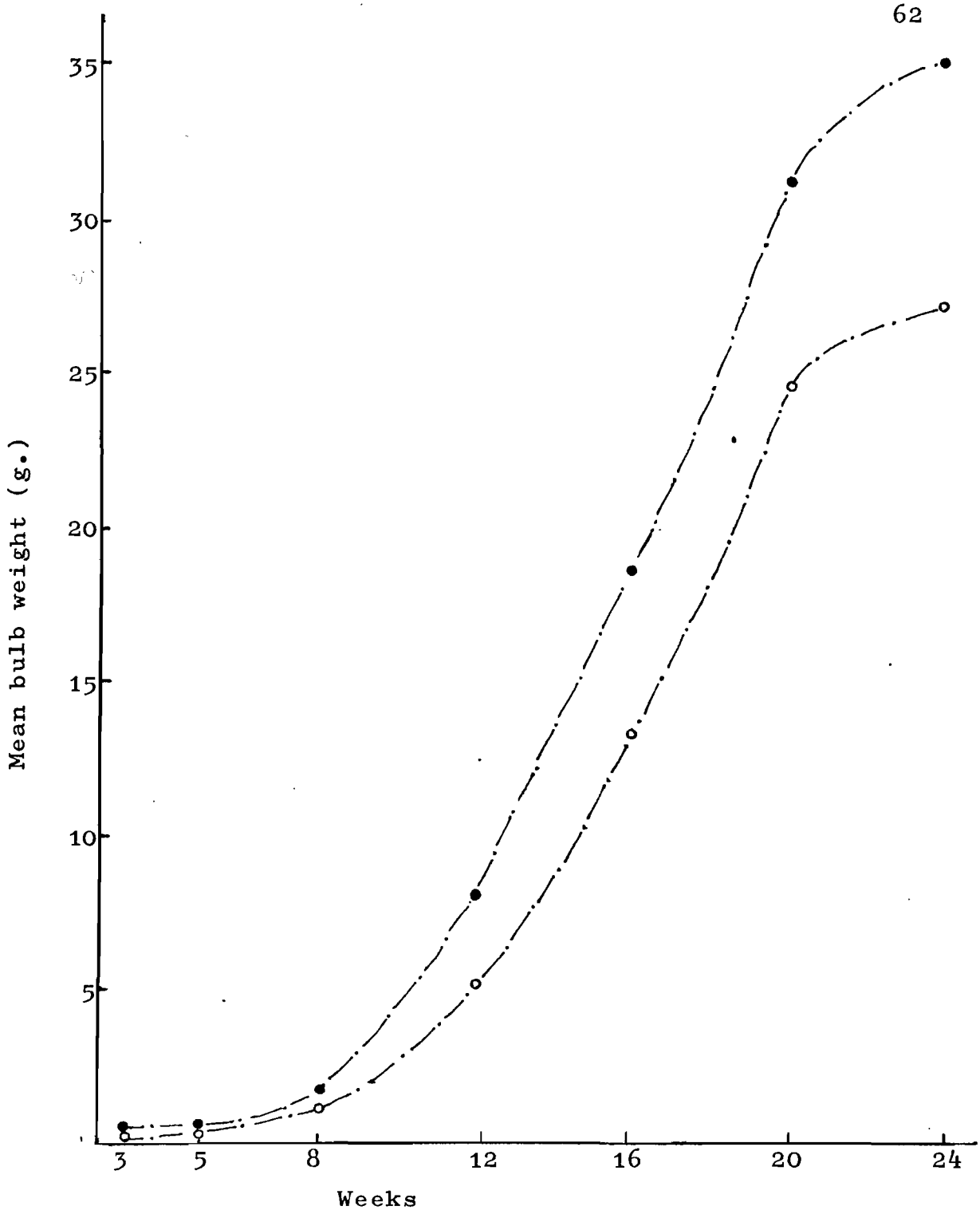


Figure 8. Bulb weight of onions (Field Conditions).

● With Endogone

o Without Endogone

—·—Unsterilised soil

Table 6. Statistical Analysis. Comparing Mycorrhizal and non-Mycorrhizal onions (5 replicates)
S = Significant difference; I = Insignificant difference; End. = Endogone

Weeks after trans-planting	Soil treatment	Mean height(cm) Controlled Conditions +End.	Mean height(cm) Controlled Conditions -End.	Analysis of Variance(5%)	Bulb weight(g) Field Conditions +End.	Bulb weight(g) Field Conditions -End.	Analysis of Variance(5%
3	Sterilised	7.003^{A}	8.020^{C}	A Vs B: I; A Vs C: I;			
	Unsterilised	8.300^{B}	8.100^{D}	B Vs D: I; C Vs D: I;	0.300^{F}	0.185^{H}	F Vs H: I;
5	Sterilised	12.800^{A}	12.004^{C}	A Vs B: I; A Vs C: I;			
	Unsterilised	10.500^{B}	11.503^{D}	B Vs D: I; C Vs D: I;	0.470^{F}	0.318^{H}	F Vs H: I;
8	Sterilised	21.406^{A}	16.100^{C}	A Vs B: I; A Vs C: I;			
	Unsterilised	17.325^{B}	17.077^{D}	B Vs D: I; C Vs D: I;	1.445^{F}	1.178^{H}	F Vs H: I;
12	Sterilised	36.056^{A}	25.184^{C}	A Vs B: S; A Vs C: S;			
	Unsterilised	27.043^{B}	24.922^{D}	B Vs D: I C Vs D: I;	7.950^{F}	5.135^{H}	F Vs H: I;
16	Sterilised	43.165^{A}	30.036^{C}	A Vs B: S; A Vs C: S;			
	Unsterilised	33.068^{B}	30.462^{D}	B Vs D: I; C Vs D: I;	18.558^{F}	13.430^{H}	F Vs H: I;
20	Sterilised	46.758^{A}	34.224^{C}	A Vs B: S; A Vs C: S;			
	Unsterilised	27.545^{B}	34.853^{D}	B Vs D: S; C Vs D: I;	32.108^{F}	24.448^{H}	F Vs H: S;
24	Unsterilised				35.165^{F}	27.818^{H}	F Vs H: S;

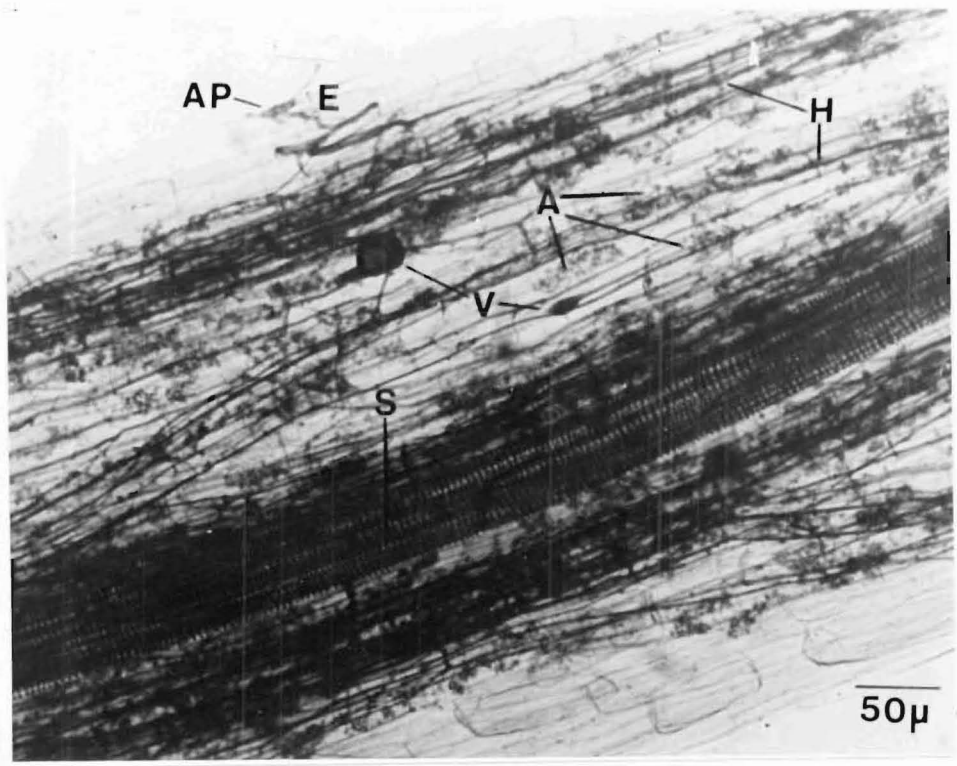


Figure 9. Onion root segment colonised by Endogone

A = arbuscules

AP = appressorium

E = entry point

H = hyphae

V = vesicles

S = stele

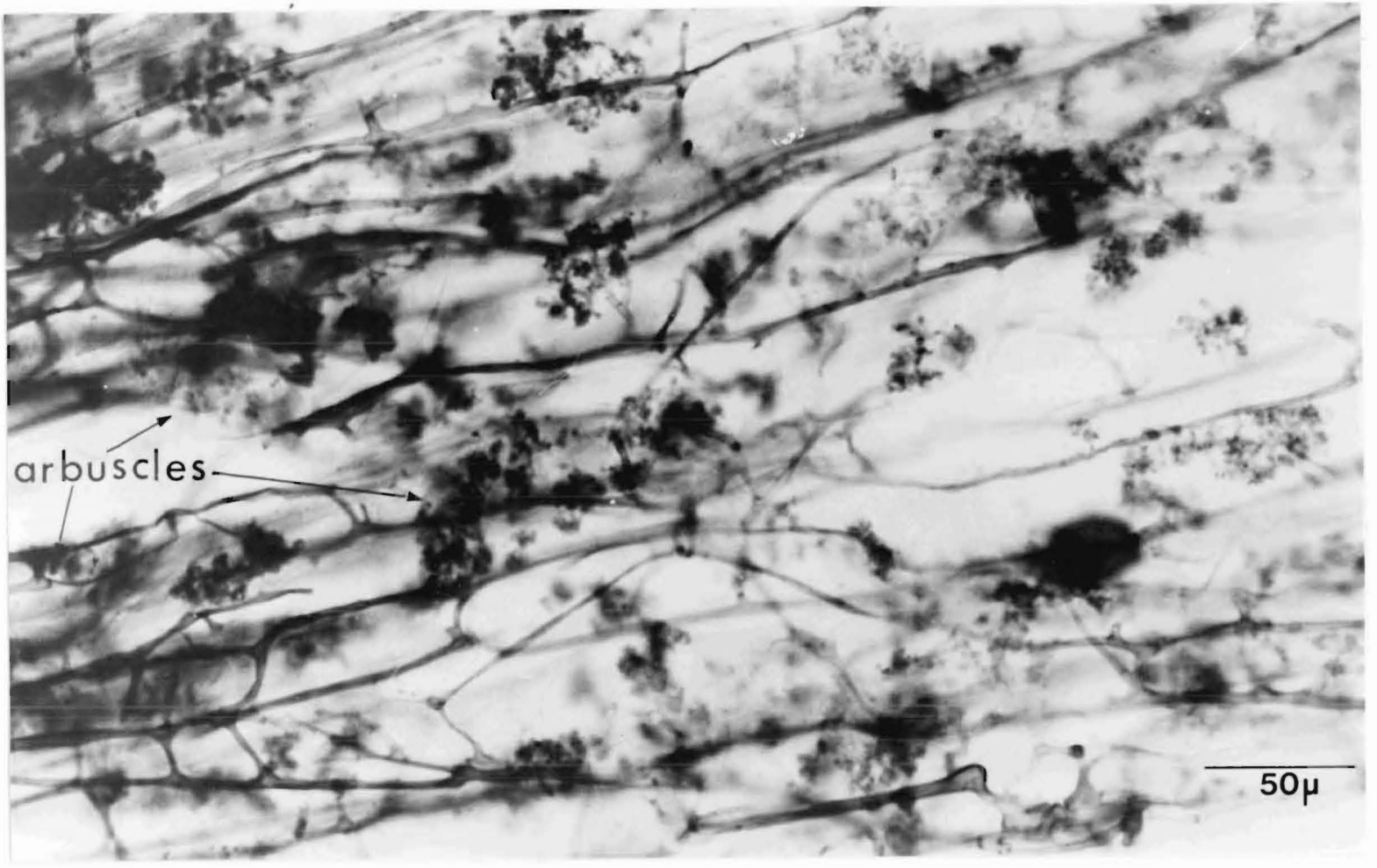


Figure 10a. Endogone hyphae and arbuscules in onion root segment

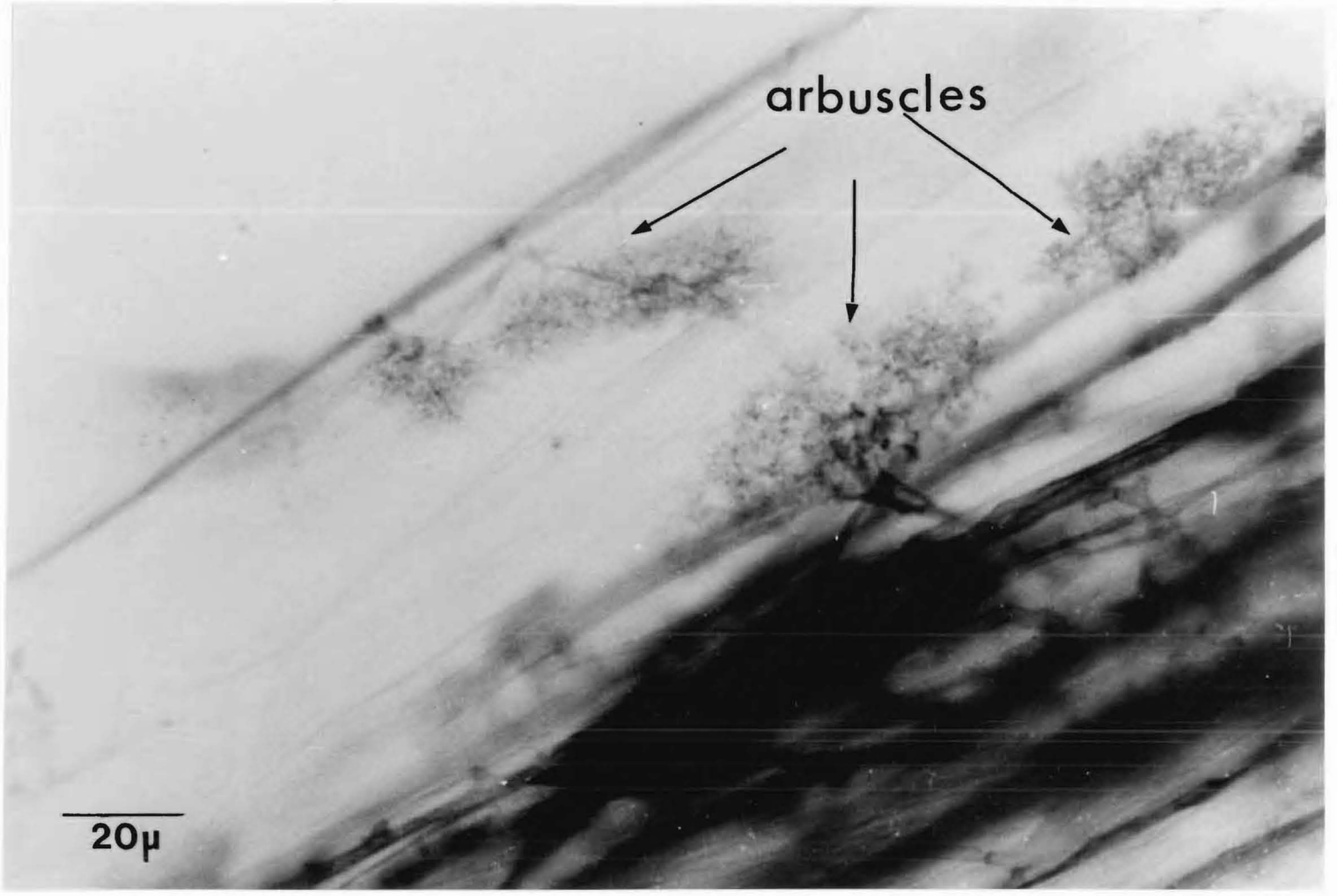


Figure 10b. Endogone hyphae and arbuscules in onion root segment

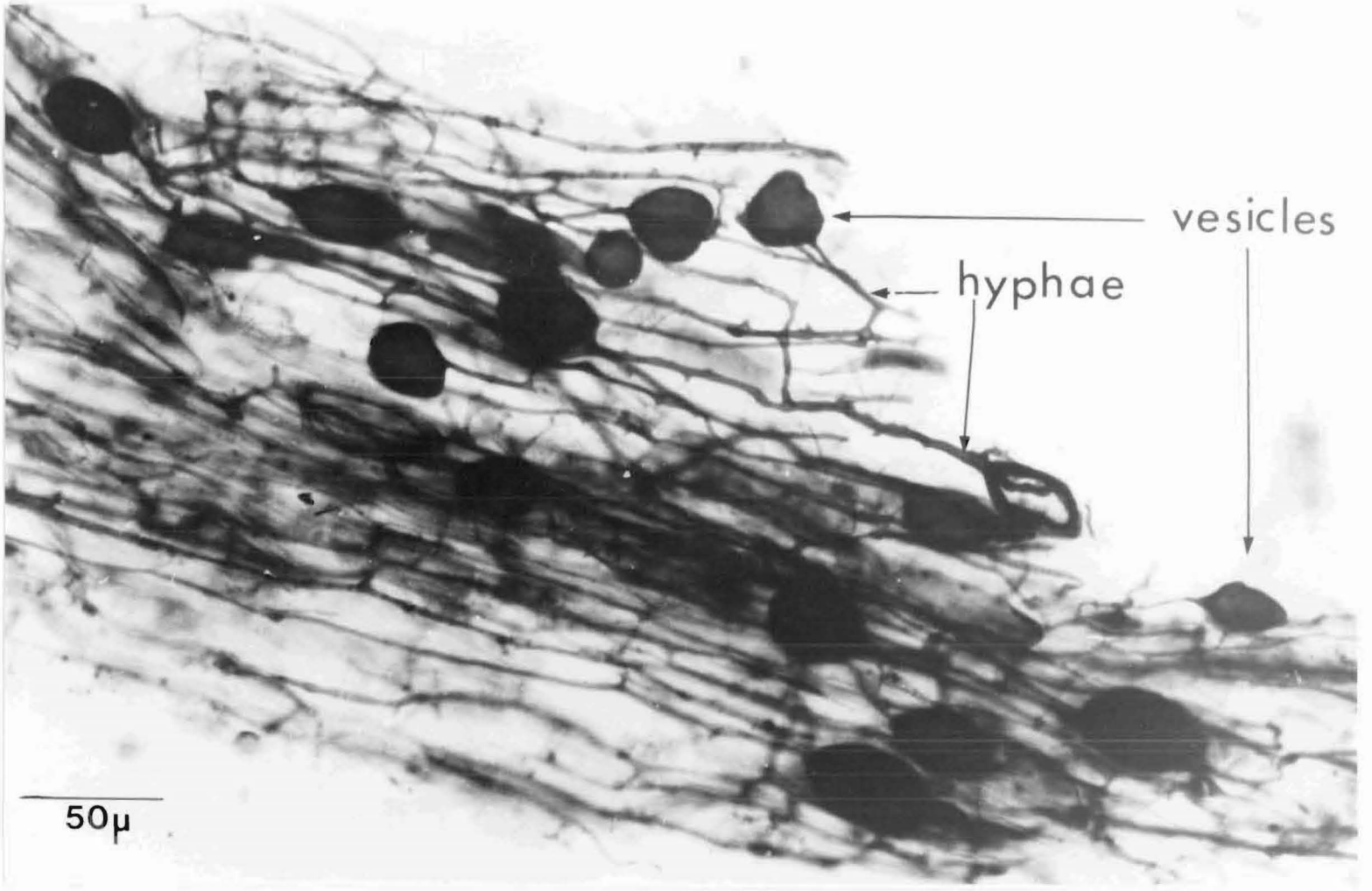


Figure 11. Endogone hyphae and vesicles in onion root segment

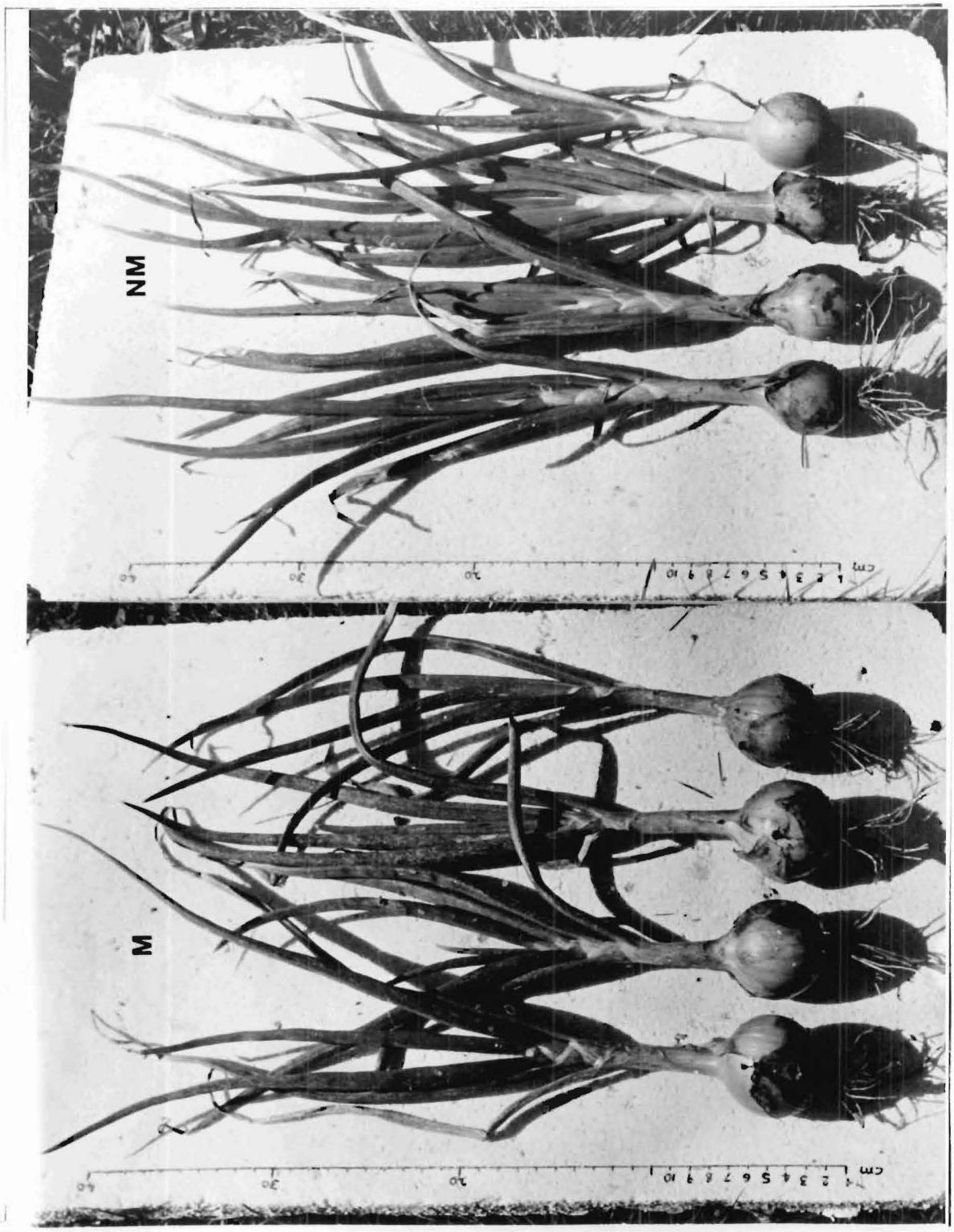


Figure 12. Twelve week old mycorrhizal (M) and non-mycorrhizal (NM) onion plants

1.2. Effect of Endogone on A. porrum growth

1.2.1. Percentage of root colonisation by Endogone.

In plants treated with Endogone spores, root colonisation was seen to be initiated at the 5th week of harvest. After this, the rate of colonisation increased very rapidly until the 12th week when there was a slight levelling off as shown in figure 13. However, there appeared to be a slight increase again after the 16th week of plants grown in unsterilised soil under both controlled and field conditions before levelling off at the 20th week. As high as 68% root colonisation was achieved.

Under controlled conditions, root colonisation was significantly greater in plants grown in sterilised soil than in unsterilised soil after 12 weeks, as shown in Table 7. Control plants did not develop mycorrhizal associations, except in two instances, shown at the 8th and 16th week harvest, as shown in figure 13.

Under field conditions, root colonisation by Endogone was significantly greater than those placed in growth rooms, reaching a maximum of 78% root colonisation at the 28th week harvest. Mycorrhizal development occured in the controls but were of low incidence and did not exceed 6%.

1.2.2. Effect of Endogone on dry weight of leek root system and number of roots per leek plant.

Under controlled conditions, control plants (Figure 14) showed very similar root growth patterns, rising rapidly after 12 weeks, starting to level off at 24 weeks. Those

inoculated with Endogone showed considerably greater root growth, with those grown in sterile soil being significantly greater in root growth than those in unsterile soil (Table 8).

Similarly, Endogone treated plants under field conditions exhibited significantly greater root growth than their control counterparts (Table 9). Generally, plants grown in the field yielded greater root growth than those placed in the growth rooms.

1.2.3. Effect of Endogone on dry weight of leek mycorrhizal roots. The difference in the dry weight of mycorrhizal root aspect under controlled conditions became clear at the 12th week harvest after which the rates of increase in plants given Endogone were very rapid as shown in figure 15. Again, the increase was significantly greater in those grown in sterile soil than those in unsterile soil as shown in Table 10.

Plants inoculated with Endogone in the field resulted in a recording of (1.613 g.) dry weight mycorrhizal roots which almost doubled that in the plants grown in the growth room (0.839 g.) at the 24th week harvest.

1.2.4. Effect of Endogone on mean height of leek plant. Leeks grown in the presence of Endogone were significantly taller than those given only the leachings (Table 11) under controlled conditions, with those grown in sterilised soil being taller than those in unsterilised soil as shown in figure 16.

Endogone treated plants were similarly taller in the field and these were taller than those grown under controlled conditions as shown in Table 12.

1.2.5. Effect of Endogone on dry weight per leek plant. Endogone treated leeks were heavier than control plants, but these differences were significant only after the 16th week. This was shown in leeks grown under controlled (Table 11) and field (Table 12) conditions. The 16th week harvest also showed that Endogone treated plants grown in sterilised soil under controlled conditions were significantly heavier than those grown in unsterilised soil, as shown in Table 11.

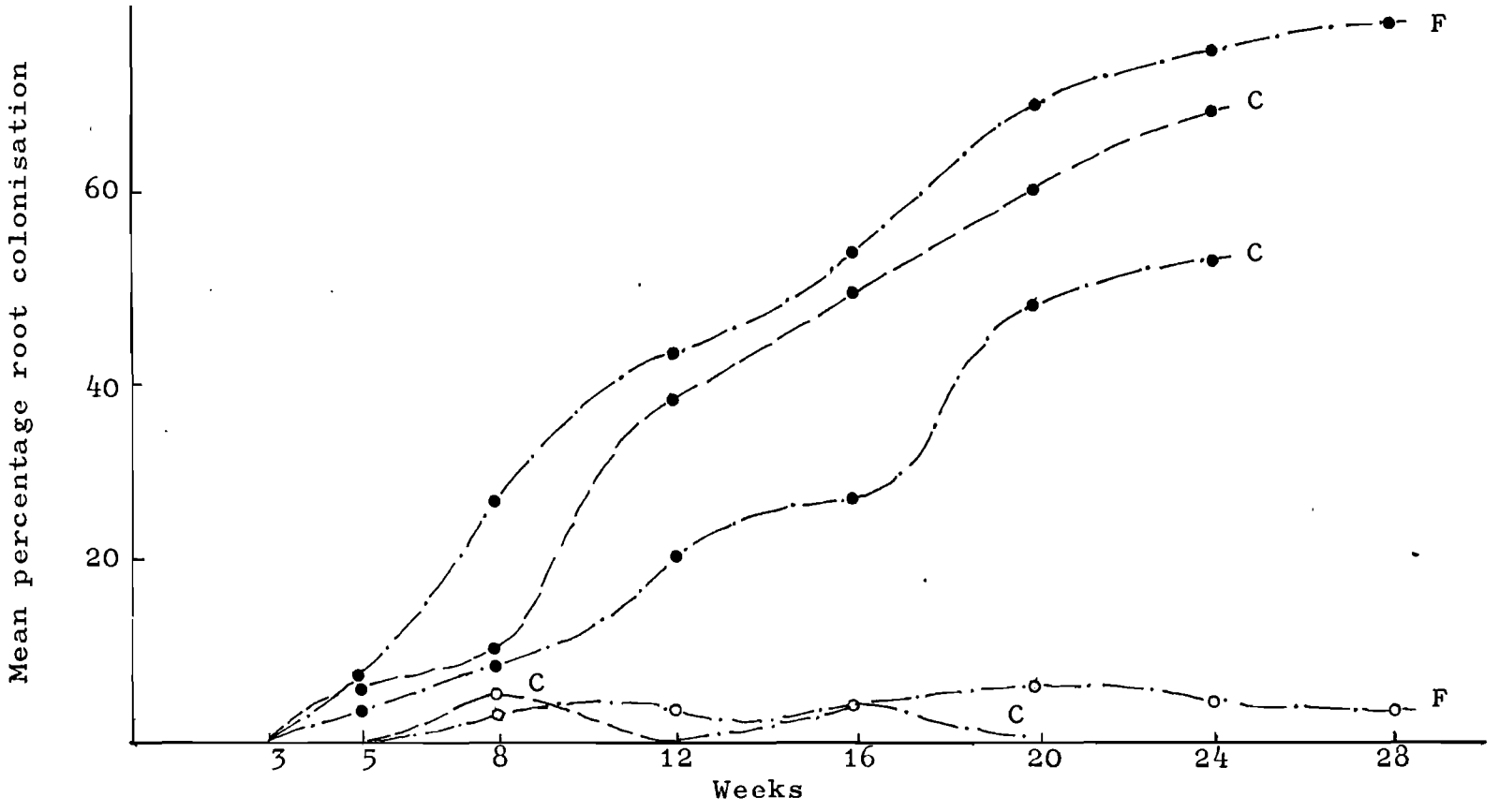


Figure 13. Percentage of roots of leeks colonised by Endogone.

● With Endogone; ○ Without Endogone;

——Sterilised soil; —·—Unsterilised soil;

C = Controlled Conditions; F = Field Conditions

Table 7. Statistical Analysis. Comparing leek root colonisation by Endogone (End.) under Controlled and Field Conditions (5 replicates).

Weeks after trans-planting	Soil treatment	% root colonisation per plant (back-transformed) Controlled +End.	Controlled -End.	Field +End.	Field -End.	Analysis of Variance (5%) S...significant difference I...Insignificant difference
3	Sterilised	1.000^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.000^{B}	1.000^{D}	1.000^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
5	Sterilised	7.003^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	4.001^{B}	1.000^{D}	8.002^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
8	Sterilised	10.998^{A}	5.999^{C}			A Vs B: I; A Vs C: I; B Vs D: S;
	Unsterilised	9.003^{B}	1.000^{D}	27.002^{F}	4.001^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
12	Sterilised	38.003^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	21.003^{B}	1.000^{D}	42.997^{F}	4.001^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
16	Sterilised	48.997^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	27.002^{B}	4.999^{D}	53.995^{F}	4.999^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
20	Sterilised	60.997^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	48.000^{B}	1.000^{D}	70.006^{F}	7.003^{H}	B Vs F: S; D Vs H: S; F Vs H: S;
24	Sterilised	69.009^{A}	1.000^{C}			A Vs B: S; A Vs C: S; B Vs D: S;
	Unsterilised	53.005^{B}	1.000^{D}	75.999^{F}	4.999^{H}	B Vs F: S; D Vs H: S; F Vs H: S;
28	Unsterilised			79.000^{F}	4.001^{H}	F Vs H: S;

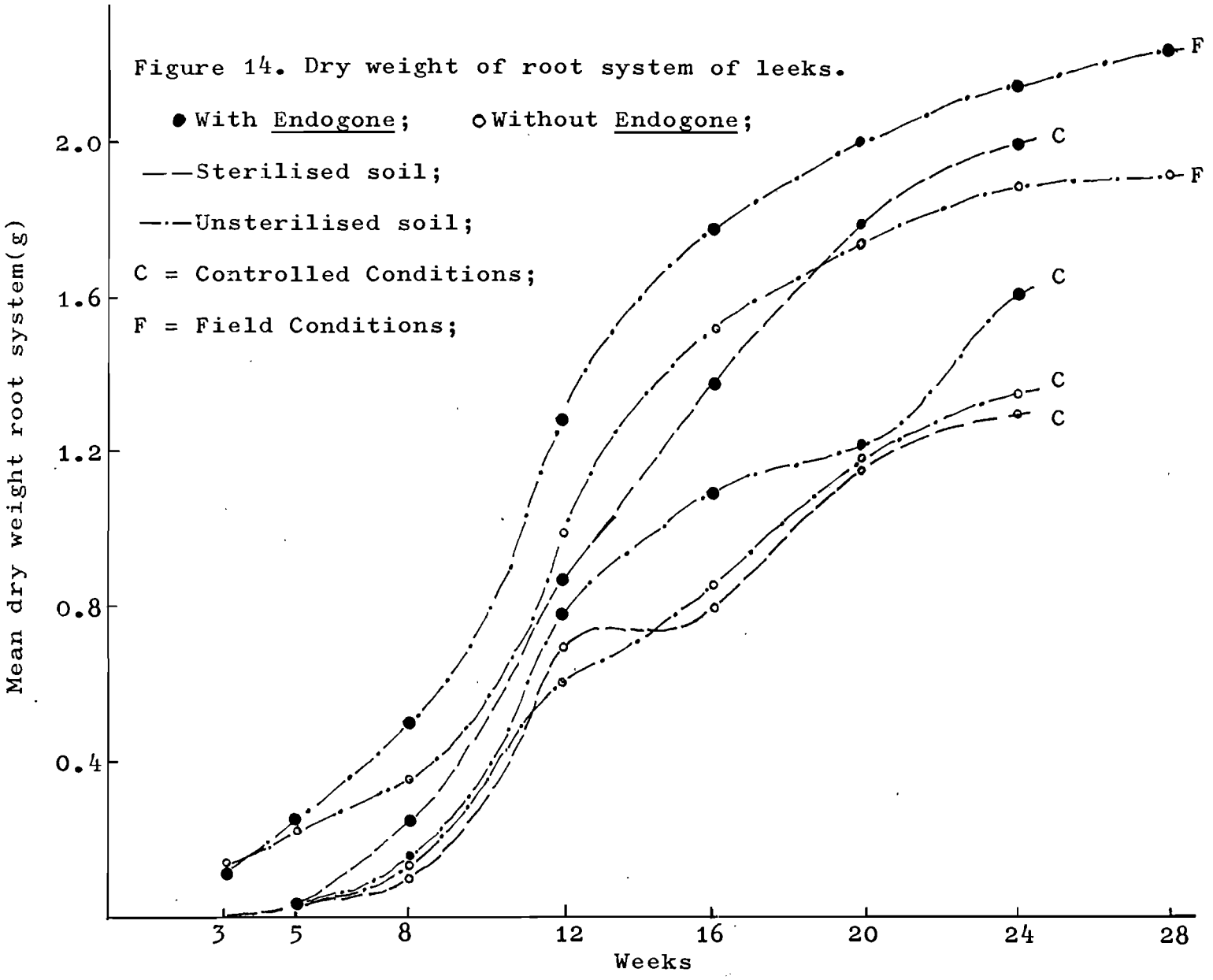


Figure 14. Dry weight of root system of leeks.
● With Endogone; ○ Without Endogone;
——Sterilised soil;
—·—Unsterilised soil;
C = Controlled Conditions;
F = Field Conditions;
Mean dry weight root system(g)
2.0
1.6
1.2
0.8
0.4
3
5
8
12
16
20
24
28
Weeks
F
C
F
C
C
C

Table 8 . Statistical Analysis. Comparing Mycorrhizal and non-Mycorrhizal leeks (5replicates) grown under Controlled Conditions. End.=Endogone; S=Significant difference; I=Insignificant difference;

Weeks after trans-planting	Soil treatment	Dry weight root system per plant(g) +End.	-End.	Analysis of Variance(5%)	Number of roots per plant(g) +End.	-End.	Analysis of Variance(5%)
3	Sterilised	0.026^{A}	0.028^{C}	A Vs B: I; A Vs C: I;	10^{A}	7^{C}	A Vs B: I; A Vs C: I;
	Unsterilised	0.028^{B}	0.027^{D}	B Vs D: I; C Vs D: I;	6^{B}	8^{D}	B Vs D: I; C Vs D: I;
5	Sterilised	0.033^{A}	0.036^{C}	A Vs B: I; A Vs C: I;	16^{A}	19^{C}	A Vs B: I; A Vs C: I;
	Unsterilised	0.035^{B}	0.032^{D}	B Vs D: I; C Vs D: I;	11^{B}	10^{D}	B Vs D: I; C Vs D: I;
8	Sterilised	0.190^{A}	0.144^{C}	A Vs B: I; A Vs C: I;	25^{A}	22^{C}	A Vs B: I; A Vs C: I;
	Unsterilised	0.163^{B}	0.151^{D}	B Vs D: I; C Vs D: I;	20^{B}	15^{D}	B Vs D: I; C Vs D: I;
12	Sterilised	0.868^{A}	0.679^{C}	A Vs B: I; A Vs C: I;	35^{A}	28^{C}	A Vs B: I; A Vs C: I;
	Unsterilised	0.784^{B}	0.635^{D}	B Vs D: I; C Vs D: I;	30^{B}	22^{D}	B Vs D: S; C Vs D: I;
16	Sterilised	1.383^{A}	0.804^{C}	A Vs B: S; A Vs C: S;	50^{A}	33^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	1.092^{B}	0.857^{D}	B Vs D: I; C Vs D: I;	44^{B}	30^{D}	B Vs D: S; C Vs D: I;
20	Sterilised	1.084^{A}	1.165^{C}	A Vs B: S; A Vs C: S;	66^{A}	40^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	1.227^{B}	1.202^{D}	B Vs D: I; C Vs D: I;	54^{B}	38^{D}	B Vs D: S; C Vs D: I;
24	Sterilised	2.010^{A}	1.308^{C}	A Vs B: S; A Vs C: S;	80^{A}	59^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	1.613^{B}	1.347^{D}	B Vs D: S; C Vs D: I;	71^{B}	52^{D}	B Vs D: S; C Vs D: S;

Table 9 . Statistical Analysis.

Comparing Mycorrhizal and non-Mycorrhizal leeks (5 replicates) grown under Field Conditions.

End. = Endogone; S = Significant difference; I = Insignificant difference;

Weeks after trans-planting	Dry weight root system per plant(g) +End. (F)	-End. (H)	Analysis of Variance (5%)	Number of roots per plant +End. (F)	-End. (H)	Analysis of Variance (5%)
3	0.110	0.130	F Vs H: I;	13	10	F Vs H: I;
5	0.247	0.230	F Vs H: I;	46	3	F Vs H: I;
8	0.506	0.372	F Vs H: I;	72	63	F Vs H: S;
12	1.290	1.005	F Vs H: S;	125	109	F Vs H: S;
16	1.783	1.527	F Vs H: S;	208	186	F Vs H: S;
20	2.016	1.758	F Vs H: S;	273	241	F Vs H: S;
24	2.150	1.886	F Vs H: S;	316	277	F Vs H: S;
28	2.238	1.920	F Vs H: S;	335	289	F Vs H: S;

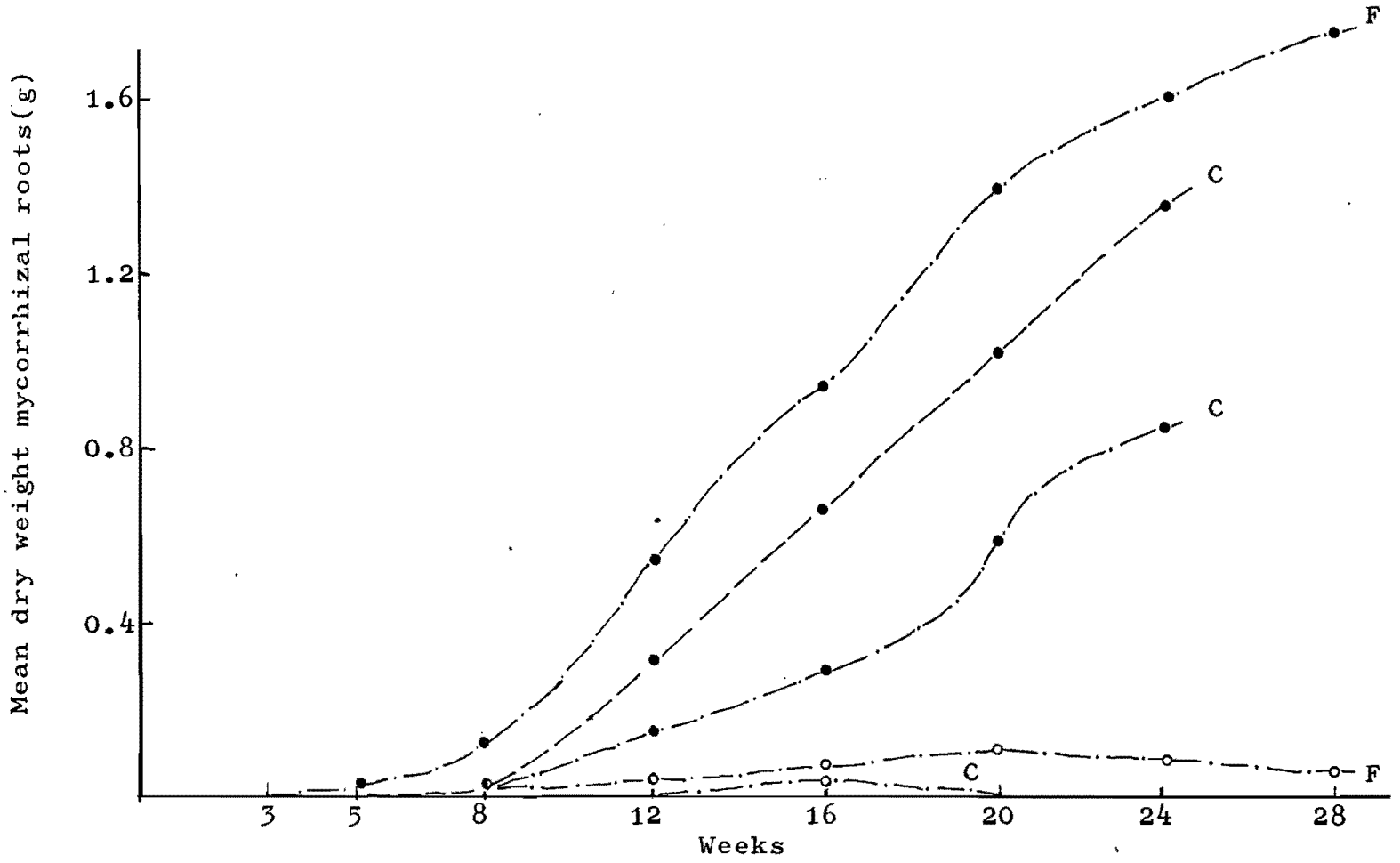


Figure 15. Dry weight of mycorrhizal roots of leeks.

● With Endogone; ○ Without Endogone;

——Sterilised soil; —·—Unsterilised soil;

C = Controlled Conditions; F = Field Conditions;

Table 10. Statistical Analysis. Comparing leek dry weight mycorrhizal roots caused by Endogone(End.) under Controlled and Field Conditions (5 replicates).

Weeks after trans-planting	Soil treatment	Dry weight mycorrhizal roots per plant (Back-transformed) Controlled +End.	Controlled -End.	Field +End.	Field -End.	Analysis of Variance (5%) S...Significant difference I...Insignificant difference
3	Sterilised	1.000^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.000^{B}	1.000^{D}	1.000^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
5	Sterilised	1.003^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.010^{B}	1.000^{D}	1.017^{F}	1.000^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
8	Sterilised	1.024^{A}	1.001^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.013^{B}	1.000^{D}	1.031^{F}	1.011^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
12	Sterilised	1.321^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.156^{B}	1.000^{D}	1.542^{F}	1.030^{H}	B Vs F: I; D Vs H: I; F Vs H: I;
16	Sterilised	1.664^{A}	1.000^{C}			A Vs B: I; A Vs C: I; B Vs D: I;
	Unsterilised	1.283^{B}	1.034^{D}	1.945^{F}	1.061^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
20	Sterilised	2.083^{A}	1.000^{C}			A Vs B: I; A Vs C: S; B Vs D: I;
	Unsterilised	1.577^{B}	1.000^{D}	2.391^{F}	1.105^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
24	Sterilised	2.366^{A}	1.000^{C}			A Vs B: I; A Vs C: S; B Vs D: S;
	Unsterilised	1.339^{B}	1.000^{D}	2.608^{F}	1.076^{H}	B Vs F: S; D Vs H: I; F Vs H: S;
28	Unsterilised			2.750^{F}	1.058^{H}	F Vs H: S;

Figure 16. Mean height of leek plants

● With Endogone; ○ Without Endogone;

——Sterilised soil; —·—Unsterilised soil;

C = Controlled Conditions;

F = Field Conditions;

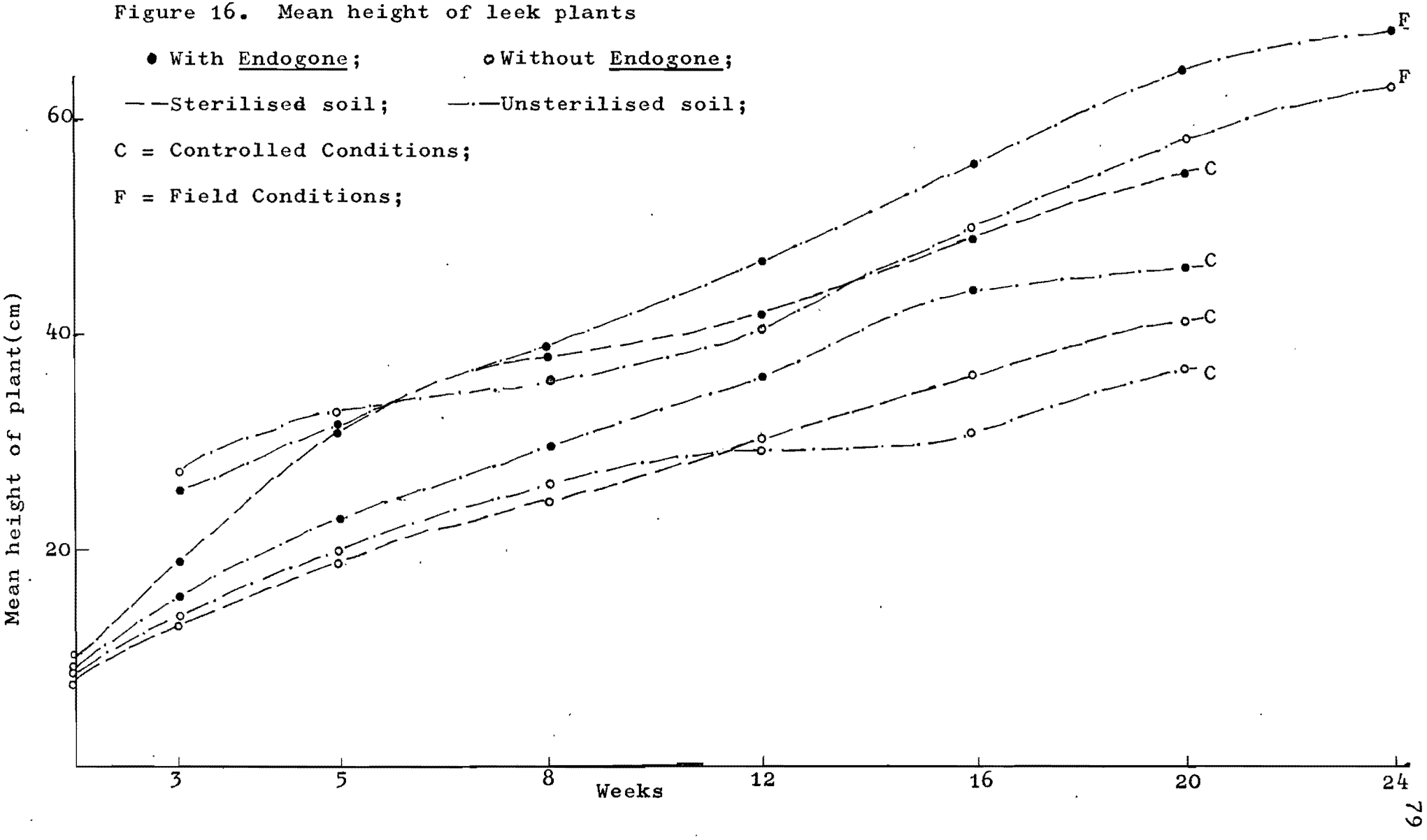


Table 11. Statistical Analysis. Comparing Mycorrhizal and non-Mycorrhizal leeks (5 replicates) grown under Controlled Conditions. End.=Endogone; S=Significant difference; I=Insignificant difference;

Weeks after trans-planting	Soil treatment	Mean dry weight shoot system (gms) +End.	-End.	Analysis of Variance(5%)	Average height (cm) +End.	-End.	Analysis of Variance(5%)
3	Sterilised	0.009^{A}	0.009^{C}	A Vs B: I; A Vs C: I;	9.945^{A}	8.027^{C}	A Vs B: I: A Vs C: I;
	Unsterilised	0.009^{B}	0.008^{D}	B Vs D: I; C Vs D: I;	8.786^{B}	8.350^{D}	B Vs D: I; C Vs D: I;
5	Sterilised	0.026^{A}	0.018^{C}	A Vs B: I; A Vs C: I;	19.150^{A}	13.182^{C}	A Vs B: I; A Vs C: S;
	Unsterilised	0.020^{B}	0.016^{D}	B Vs D: I; C Vs D: I;	15.652^{B}	13.900^{D}	B Vs D: I; C Vs D: I;
8	Sterilised	0.110^{A}	0.062^{C}	A Vs B: I; A Vs C: I;	31.095^{A}	19.066^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	0.100^{B}	0.075^{D}	B Vs D: I; C Vs D: I;	23.540^{B}	20.738^{D}	B Vs D: I; C Vs D: I;
12	Sterilised	0.400^{A}	0.284^{C}	A Vs B: I; A Vs C: I;	38.563^{A}	24.684^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	0.328^{B}	0.275^{D}	B Vs D: I; C Vs D: I;	29.597^{B}	26.235^{D}	B Vs D: I; C Vs D: I;
16	Sterilised	0.914^{A}	0.390^{C}	A Vs B: S; A Vs C: S;	42.694^{A}	29.903^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	0.608^{B}	0.382^{D}	B Vs D: S; C Vs D: I;	36.042^{B}	29.247^{D}	B Vs D: S; C Vs D: I;
20	Sterilised	1.620^{A}	0.761^{C}	A Vs B: S; A Vs C: S;	49.771^{A}	35.880^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	0.900^{B}	0.790^{D}	B Vs D: I; C Vs D: I;	43.210^{B}	30.755^{D}	B Vs D: S; C Vs D: I;
24	Sterilised	2.005^{A}	1.135^{C}	A Vs B: S; A Vs C: S;	55.833^{A}	41.066^{C}	A Vs B: S; A Vs C: S;
	Unsterilised	1.736^{B}	1.019^{D}	B Vs D: S; C Vs D: I;	46.288^{B}	36.717^{D}	B Vs D: S; C Vs D; S;

Table 12. Statistical Analysis.

Comparing Mycorrhizal and non-Mycorrhizal leeks (5 replicates) grown under Field Conditions.

End. = Endogone; S = Significant difference; I = Insignificant difference;

Weeks after transplanting	Mean Dry weight shoot system (g.) +End. (F)	-End. (H)	Analysis of Variance (5%)	Average height (cm) +End. (F)	-End. (H)	Analysis of Variance (5%)
5	2.735	3.015	F Vs H: I;	25.450	27.530	F Vs H: I;
8	8.443	9.363	F Vs H: I;	31.260	32.960	F Vs H: I;
12	16.310	14.803	F Vs H: I;	38.780	35.530	F Vs H: I;
16	27.090	21.980	F Vs H: S;	46.880	40.640	F Vs H: S;
20	41.058	33.915	F Vs H: S;	55.930	49.794	F Vs H: S;
24	56.993	45.635	F Vs H: S;	64.491	57.924	F Vs H: S;
28	64.483	55.043	F Vs H: S;	68.133	63.051	F Vs H: S;

2. DISCUSSION

Plants inoculated with Endogone showed greater growth rates than control plants (Figure 12), indicating that in soils deficient in phosphate, induced v.a. mycorrhiza is advantageous to the host plant.

2.1. V.A. mycorrhizal development

The results obtained indicate that v.a. mycorrhizal development tended to follow a three phase pattern, namely a lag phase, followed immediately by a phase of extensive mycorrhizal development which gradually tapers off into a levelling-off phase. This pattern was evident in plants grown under controlled and field conditions and whether mycorrhizae were estimated as percentage root colonised or as dry weight of mycorrhizal roots. This has also been shown by other quantitative studies of mycorrhizae at successive stages of host development. Otto (1962) found that during three successive years after sowing, the percentage of roots of apples infected was low to begin with, but increased progressively to a maximum of 80 - 100%. In tobacco seedlings, Peuss (1958) observed that root colonisation increased dramatically one week after transplanting to reach a maximum of 70 - 80% three weeks later. Sutton (1973) recorded 0.6% colonisation by Endogone three weeks after sowing beans, but later observed maximum levels ranging from 40 - 84%.

The lag phase, where root colonisation is low, probably represents the time required for the Endogone

spores to put forth germ tubes, penetrate the host and then to colonise the host tissue. It was found that onions grown under controlled conditions were colonised earlier (3 weeks after inoculation) than those grown in the field (5 weeks after inoculation). The former were grown and inoculated in sterile soil and the latter in unsterile soil. It may have been that the Endogone introduced into the unsterile soil had first to establish itself in the soil undergoing competition and antagonism with the indigenous micro-organisms, while the Endogone placed in the sterile soil would probably not have undergone as long a period of establishment. Eight weeks after transplanting, mycorrhizal development increased dramatically, the rate climbing rapidly until the 16th week (Figures 2 and 4). This marked increase during the extension phase may be caused by the introduced Endogone having established mycelia in the soil and producing penetration structures to initiate and thus cause a proliferation of new colonies in the roots. In addition, this period of time also coincided with that of rapid root formation, thus increasing the availability of host tissue for the germinating Endogone spores to infect.

In the onion, the phase of prolific mycorrhizal development terminated after the 16th week, with the rate levelling off. One causal factor may be the decrease in the rate of root formation, as shown by the levelling off in the dry weight of root system (Figure 3) and in the number of roots (Figure 5), thus decreasing the availability of non-mycorrhizal roots. The fungus itself may

be undergoing physiological changes which may restrict root colonisation. In addition, there may be the accumulating presence of organisms antagonistic to Endogone. Root exudates also alter with age and those secreted at this late stage of the host's life may inhibit or restrict root colonisation. Sutton (1973) suggested that physiological changes in the host related to reproductive growth may directly or indirectly restrict the growth of Endogone in the root cortex and possibly even prevent new infections. However, in a biennial host such as the onion, the extensive phase is probably not being interrupted by such physiological changes and instead host factors associated with dormancy may restrict mycorrhizal development.

This study has been centred and largely dependent on v.a. mycorrhizal development. Mycorrhizal estimations expressed as percentage root colonised do not consider the changing dimensions of the root system and thus may not necessarily present a correct picture of the abundance of mycorrhizae in a plant (Gerdemann, 1968). Conversely, mycorrhizae estimations expressed as the dry weight mycorrhizal roots, compare absolute quantities of mycorrhizae in the host at specific stages of the growth of the host. For example, Table 2, indicates that mycorrhizal development in onions in treatment A (with Endogone in sterilised soil, under controlled conditions) was greater than in treatment F (with Endogone, in unsterilised soil, in the field). However, in Table 4, the converse is indicated and is probably more realistic

since the changing weight of the root system was taken into consideration. Not being as spatially confined as those under controlled conditions were, plants in the field tended to produce more roots, thereby increasing the availability of roots for colonisation by Endogone.

2.2. Effect of Endogone on shoot weight, root weight, number of roots, plant height and bulb weight.

Measurements of the dry weight of root systems, shoot systems and bulb, of number of roots and of mean height of plant indicate a trend which very closely follows the three phase pattern shown by mycorrhizal development, that is, the rates beginning slowly, and then rapidly climbing between the 5th and 16th weeks, before levelling off. This observation indicates that v.a. mycorrhizae was most well developed when most plant dry matter is being produced. It further suggests that v.a. mycorrhizae was extensive in the stage of host development when a large proportion of total nutrient uptake occurs, to cope with the increased rates of anabolic processes. This is in agreement with the findings of Sutton (1973). Zink (1962, 1963, 1965), working on bunching onions and spring spinach reported similar trends and observed that nutrient uptake rates corresponded closely to those of dry matter production.

The records of weight of root systems are in conflict with those reported by previous workers. Hayman and Mosse (1971) and Mosse and Hayman (1971) working on Coprosma and

onion plants found that root growth was not significantly different in mycorrhizal and non-mycorrhizal plants, though shoot growth in inoculated plants were significantly greater than in control plants. Daft and Nicolson (1966) working on tomato and Khan (1972) working on maize recorded slower growth of roots of inoculated plants. Khan attributed these observations to poorer root growth under those conditions. The results of the present study suggests that the presence of Endogone may have a pronounced effect on the morphological features of root growth, as seen in the increases in root gross production (Figure 3). Daft and Okusanya (1973) reported similar findings. They found that Endogone colonisation increased the amount of vesicular tissue in tomato, petunia and maize plants. Wilhelm (1973) reported Endogone colonised papaya tissue to be structurally and physiologically different from uncolonised papaya root tissue.

2.3. Effect of soil sterilisation

The effect of sterilising soil for experimental use is well demonstrated. Plants grown in sterilised soil showed greater rates in root colonisation, dry weight mycorrhizal root, dry weight root and shoot systems, number of roots and height of plants than those grown in unsterilised soil. This trend is more marked in mycorrhizal plants. Heating the soil increases available phosphate (Baylis, 1967). This may be the main factor contributing to the greater growth observed, rather than the influence of Endogone. However, in similar but unsterilised soil, plants inoculated with Endogone also exhibited greater rates

in the criteria mentioned above than control plants. Moreover, control plants grown in sterilised soil exhibited the lowest rates measured. Thus it would be more likely that the increased rates seen may be attributed not to the release of phosphate through the soil being autoclaved, but rather to the development of v.a. mycorrhizae. Other workers have reported similar effects of heating soil. Mosse, Hayman and Ide (1969) found the growth rates of inoculated onions and Liquidambae styraciflua grown in sterilised soil to be much greater than in unsterilised soil. Baylis (1967), working principally on Coprosma robusta observed that the mean dry weight of mycorrhizal plants grown in sterilised soil exceed that in unsterilised soil by one-third. Sterilising soil has the adverse effect of destroying the indigenous soil microflora which may contain present potential endophytes. This is probably the reason for the low rates seen in non-inoculated plants grown in sterilised soil. The adverse effect probably offsets whatever advantage there may have been with the release of available phosphate. Various investigations into soil sterilisation have reported similar deleterious effects. Kleinschmidt and Gerdemann (1972) observed that inoculated citrus seedlings grew well in soil fumigated with a 3:1 mixture of methyl bromide and chloropicrin while control plants become stunted. Clark (1969) and Ross and Harper (1970) postulated that fumigating soil cores with methyl bromide and injecting soil to a depth of 0.9 cm with chloropicrin respectively destroyed the indigenous v.a. fungi in the soil. Some workers however, have reported beneficial effects of soil sterilisation. Hayman (1970) found that

repeated applications of formalin resulted in unexpectedly high Endogone infection, probably through selective adaptation. Strawberries became heavily infected two years after soil fumigation with chlorobromopropene (Wilhelm 1959) and after treatment with the nematocide, DD (Mosse, 1973).

CHAPTER VI

EFFECT OF *ENDOGONE* ON PATHOGENESIS

1. RESULTS

1.1. Basal root rot of onion (*F. oxysporum v. cepa*)

Plants diseased by *F. oxysporum v. cepa* showed a characteristic one-sided infection of the bulb. One side of the bulb became shiny and cream-coloured and the basal portion of bulb scales and a large number of roots were destroyed. Young onion plants (3 - 5 weeks old) irrespective of being treated with *Endogone* or not showed disease symptoms when inoculated with *F. oxysporum v. cepa* (Figure 17). This occured under controlled (Table 13) and field (Table 14) conditions. Generally between 65 - 85% of these young plants became infected, although a higher percentage of plants grown in the growth room were infected (81 - 86%, Table 13) than those in the field (66 - 83%, Table 14). Among the older plants (8 weeks and older) those treated with *Endogone* exhibited a decrease in the proportion of plants showing disease symptoms. This decrease was more pronounced with older plants, that is between 68 - 76% of the 8 week old plants being infected compared with 52 - 55% in the 20 week old plants.

In the plants given only the left over leachings from spore isolations, a high incidence (65 - 90%) of disease symptoms was seen in young and old plants alike. However, there was a slight decrease in disease symptom development in the older plants.

1.2. Pink root of onion (P. terrestris)

Plants infected by P. terrestris exhibited typical pink roots, which were short and stunted. The plants themselves were stunted and showed reduced growth.

Under controlled conditions, (Table 13), 3 - 5 week old plants showed a high incidence of infection, regardless of whether they were treated with Endogone or not (Figure 18). Between 71 - 91% of the plants became infected. Under field conditions, (Table 14), 5 week old plants began to show a decreased disease incidence (only 64% being infected compared with 75% in the 3 week old plants). Older plants (8 weeks and older) exhibited an increase in disease resistance, especially in the much older plants, that is, 50 - 57% of the 20 week old plants being infected compared with 67 - 69% of the 8 week old plants. Between 70 - 90% incidence of infection was recorded in the control plants, with the older plants tending to show a very slight tendancy towards a decrease in disease infection.

1.3. Effect of Endogone on root growth in the presence of pathogen.

Under controlled conditions, Endogone treated onion plants showed greater root growth than in the controls

(Figure 3). This enhancement of root growth is again seen in Endogone treated plants which were inoculated with either F. oxysporum v. cepa or P. terrestris though in a slightly reduced manner (Figure 19). This difference in root growth rate was not significant (Table 15).

In the field, Endogone treated plants which were inoculated with F. oxysporum v. cepa and those which were not showed very similar root growth rates (Figure 20). Their rates however, were much greater than that shown by control plants which were not treated with Endogone (Table 16).

1.4. Effect of Endogone on dry weight of onion shoot system in the presence of pathogen.

Of those plants placed in the growth rooms, dry weight of shoot system per plant was greater in the Endogone treated plants (Figure 6) than those not treated with Endogone. The further inoculation of either F. oxysporum v. cepa or P. terrestris to Endogone treated plants resulted in a decrease in dry weight of shoot system per plant (Figure 21). This decrease was not significant (Table 17).

1.5. Effect of Endogone on onion bulb weight in the presence of pathogen.

This measurement was only taken off plants grown in the field.

The bulb weight of Endogone treated plants were far greater than those which were not treated with Endogone

(Figure 8). When the former were further inoculated with either F. oxysporum v. cepa or P. terrestris a decrease in bulb weight was shown. (Figure 22). But these differences were not significant (Table 18). This (decreased) weight was still greater than in those plants which were not treated with Endogone.

1.6. Percentage root colonisation by Endogone in the presence of pathogen.

Endogone treated plants which were inoculated with the pathogens but escaped disease infection showed greater percentage root colonisation by Endogone than Endogone treated plants which were not inoculated with the pathogens. This occured under both controlled (Figure 23) and field conditions (Figure 24). However, these differences were not significant (Tables 19 and 20).

1.7. Dry weight of mycorrhizal roots in the presence of pathogen.

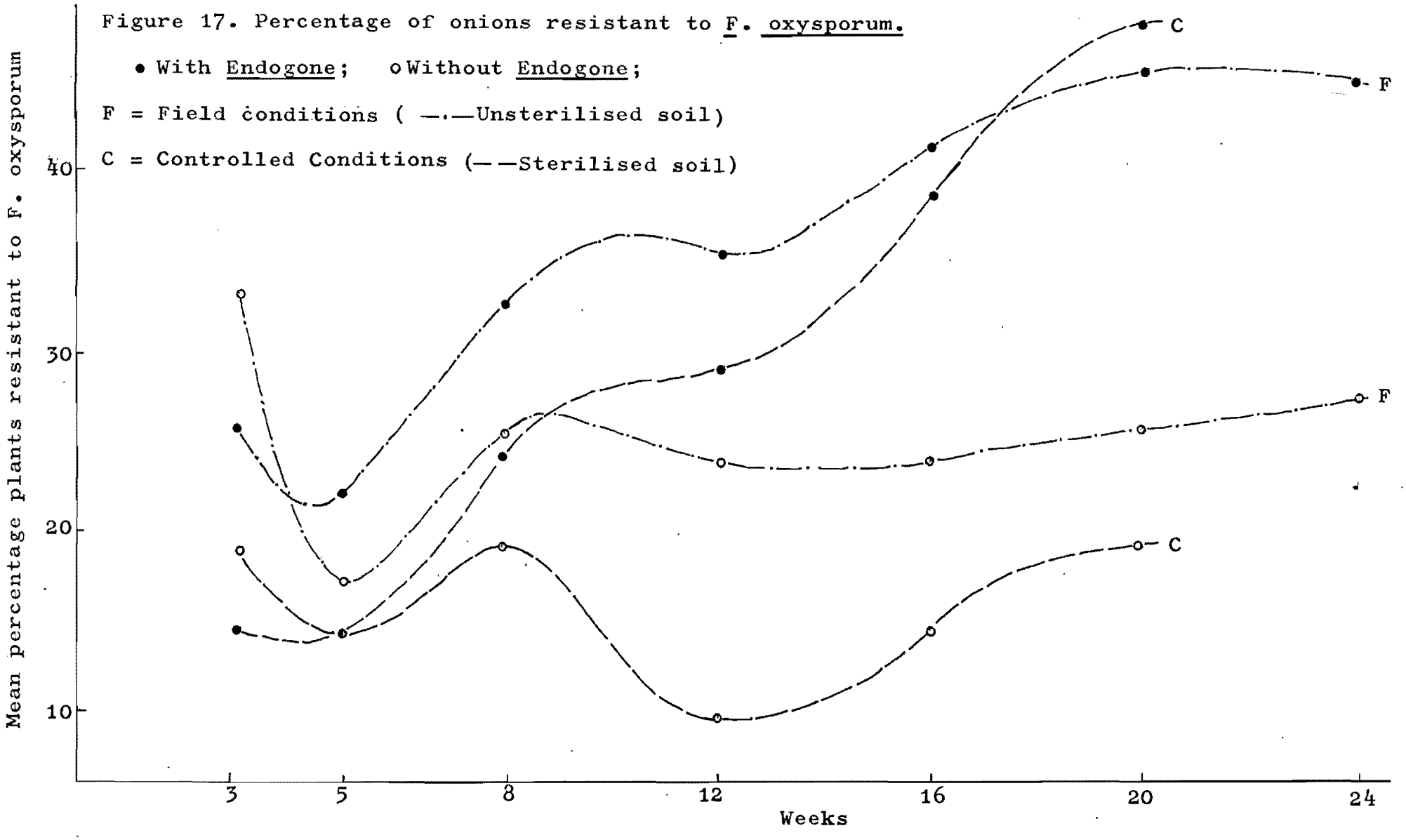
Under controlled conditions, Endogone treated plants which were inoculated with the pathogens but escaped disease infection showed lower recordings of dry weight of mycorrhizal roots than Endogone treated plants which were not inoculated with the pathogens (Figure 25). In the field, Endogone treated plants which escaped disease infection and Endogone treated plants which were not inoculated with the pathogens showed very similar trends in mycorrhizal development (Figure 26). Again, these differences were found to be insignificant (Tables 21 and 22).

Figure 17. Percentage of onions resistant to F. oxysporum.

• With Endogone; o Without Endogone;

F = Field conditions (—·—Unsterilised soil)

C = Controlled Conditions (— —Sterilised soil)



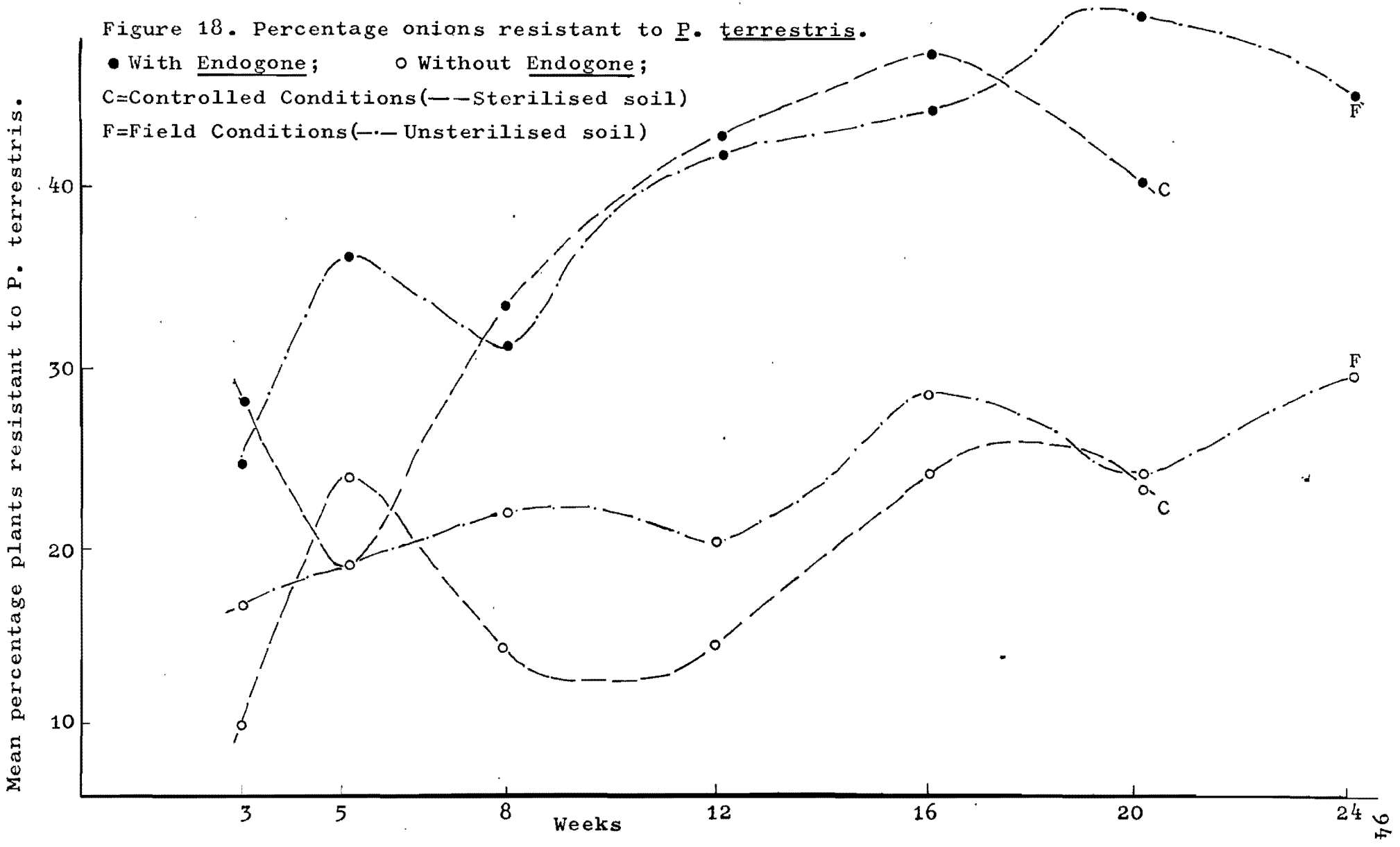


Figure 18. Percentage onions resistant to P. terrestris.
● With Endogone; ○ Without Endogone;
C=Controlled Conditions(——Sterilised soil)
F=Field Conditions(—·—Unsterilised soil)
Mean percentage plants resistant to P. terrestris.
40
30
20
10
3
5
8
12
16
20
24
Weeks
C
C
F
F

Table 13. Degree of infection by *F. oxysporum* and *P. terrestris* of plants grown under Controlled Conditions (5 replicates). End. = *Endogone*

Weeks after transplanting	Mean Percentage plants infected by particular pathogen				Analysis of Variance(5%)
	F. oxysporum		*P. terrestris*		S..Significant difference
	+End. (M)	-End. (N)	+End. (O)	-End. (P)	I..Insignificant difference
3	85.714	80.953	71.434	90.482	M Vs N: I; O Vs P: I;
5	85.774	85.714	80.953	76.191	M Vs N: I; O Vs P: I;
8	76.191	80.953	66.667	85.714	M Vs N: I; O Vs P: I;
12	71.429	90.476	57.143	85.714	M Vs N: S; O Vs P: S;
16	61.905	85.714	52.381	76.191	M Vs N: S; O Vs P: S;
20	52.381	80.952	57.143	76.191	M Vs N: S; O Vs P: S;

Table 14. Degree of infection by F. oxysporum and P. terrestris of plants grown under Field Conditions (5 replicates). End. = Endogone

Weeks after transplanting	Mean Percentage plants infected by particular pathogen				Analysis of Variance(5%) S...Significant differerence I...Insignificant difference
	F. oxysporum		P. terrestris		
	+End. (M)	-End. (N)	+End. (O)	-End. (P)	
3	74.286	65.714	75.238	83.333	M Vs N: I; O Vs P: I;
5	78.095	82.857	63.810	80.952	M Vs N: I; O Vs P: I;
8	67.619	74.762	68.571	78.095	M Vs N: I; O Vs P: I;
12	64.762	76.191	58.095	79.524	M Vs N: S; O Vs P: S;
16	59.048	76.191	55.238	71.429	M Vs N: S; O Vs P: S;
20	54.762	74.762	50.000	75.714	M Vs N: S; O Vs P: S;
24	55.238	72.857	54.286	70.476	M Vs N: S; O VS P: S;

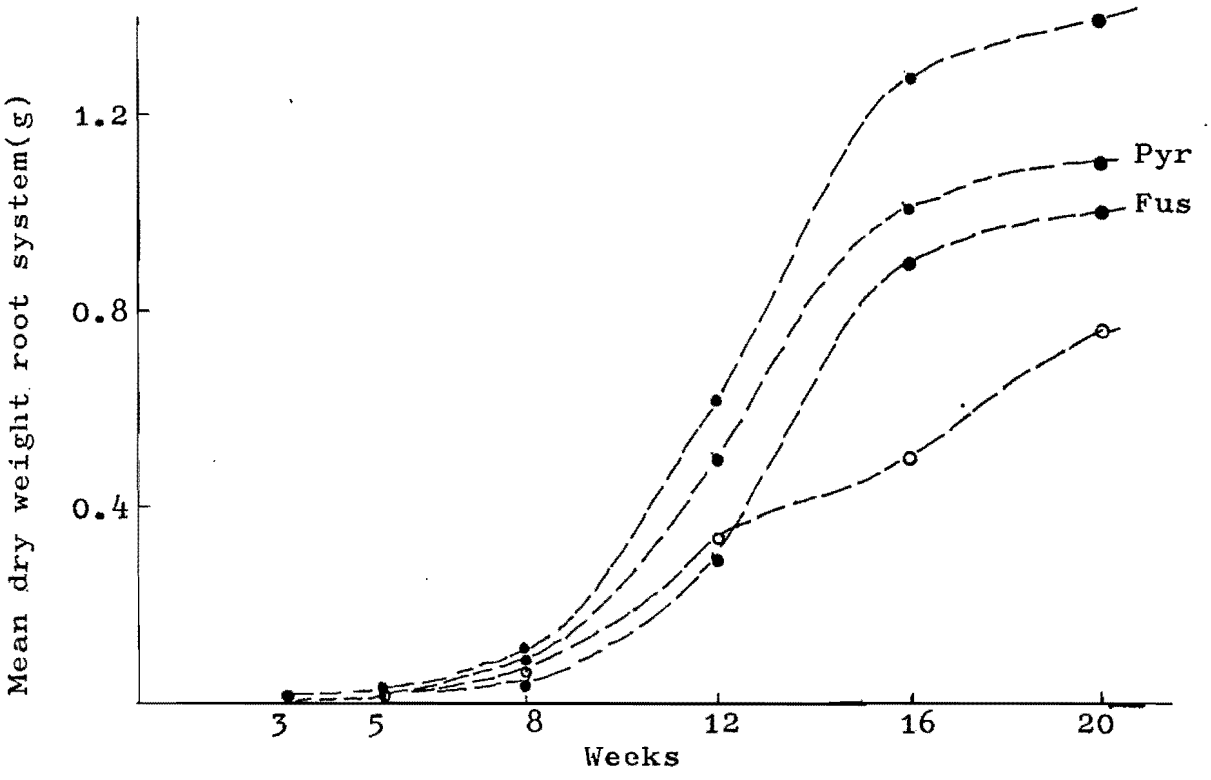


Figure 19. Dry weight of root system of onions (Controlled Conditions)

• With Endogone; o Without Endogone;

Pyr = With P. terrestris; Fus = With F. oxysporum;

— —Sterilised soil;

Table 15. Statistical Analysis.

Comparing dry weight root system as affected by Endogone (End.)

in the presence and absence of pathogen under Controlled Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after transplanting	Dry weight root system per plant (g)				Analysis of Variance(5%) S...Significant difference I...Insignificant difference			
	+End. (A)	-End. (C)	+F.oxy. +End. (X)	+P.terr. +End. (Y)				
3	0.010	0.010	0.010	0.009	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
5	0.028	0.018	0.015	0.020	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
8	0.110	0.063	0.065	0.085	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
12	0.620	0.330	0.319	0.490	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
16	1.260	0.500	0.889	1.082	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;
20	1.394	0.754	1.000	1.100	A Vs X: S;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;

Figure 20. Dry weight of root system of onions (Field Conditions)

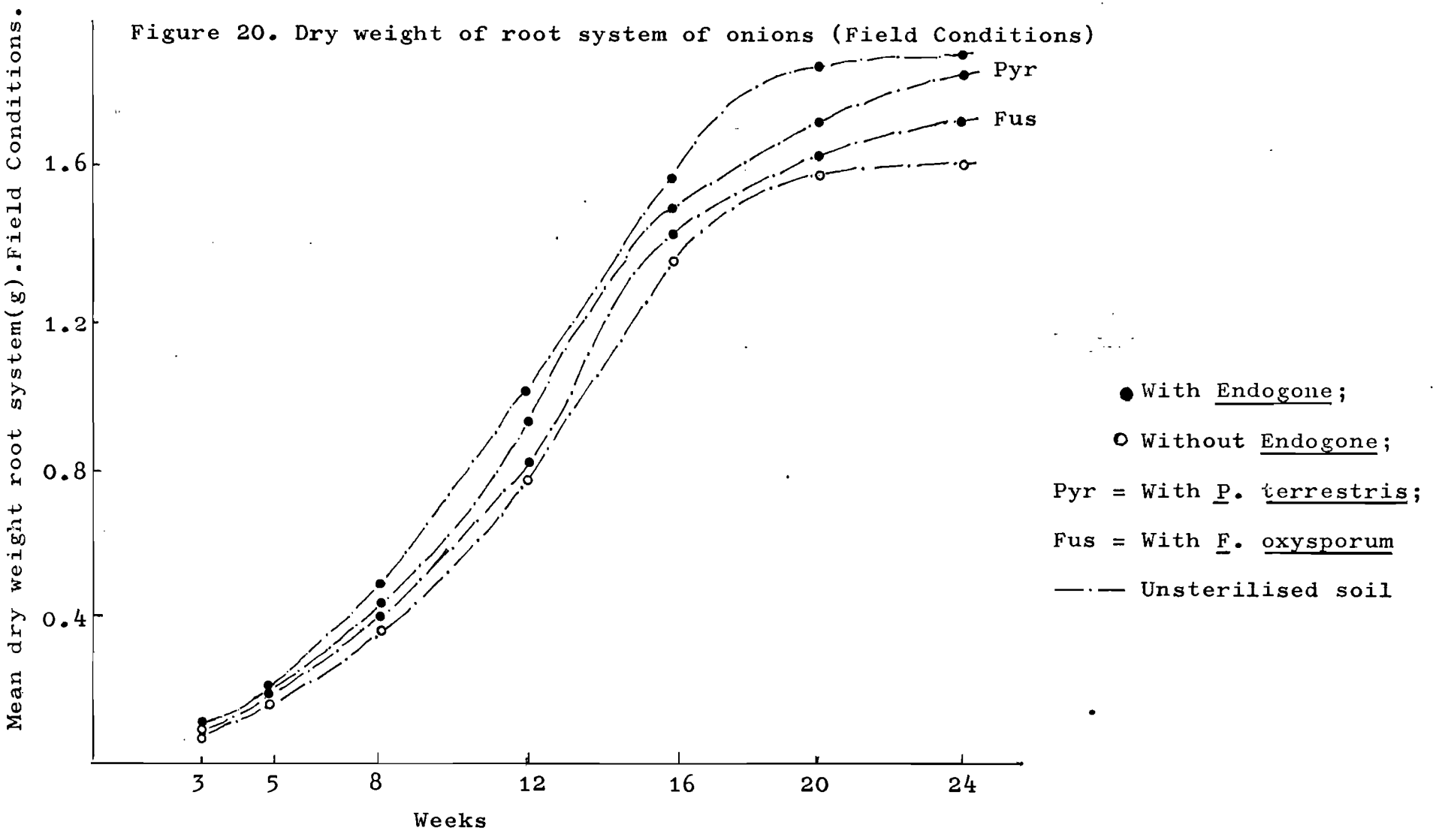


Table 16. Statistical Analysis.

Comparing dry weight root system as affected by Endogone (End.)

in the presence and absence of pathogen under Field Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after trans-planting	Dry weight root system per plant (g) +End. (F)	-End. (H)	+F.oxy. +End. (K)	+P.terr. +End. (L)	Analysis of Variance(5%) S...Significant difference I...insignificant difference
3	0.100	0.092	0.090	0.106	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
5	0.224	0.165	0.180	0.200	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
8	0.483	0.350	0.400	0.360	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
12	1.052	0.748	0.828	0.936	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
16	1.614	1.367	1.450	1.500	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
20	1.875	1.584	1.650	1.702	F Vs K: S; H Vs K: I; F Vs L: I; H Vs L: I;
24	1.926	1.625	1.744	1.865	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: S;

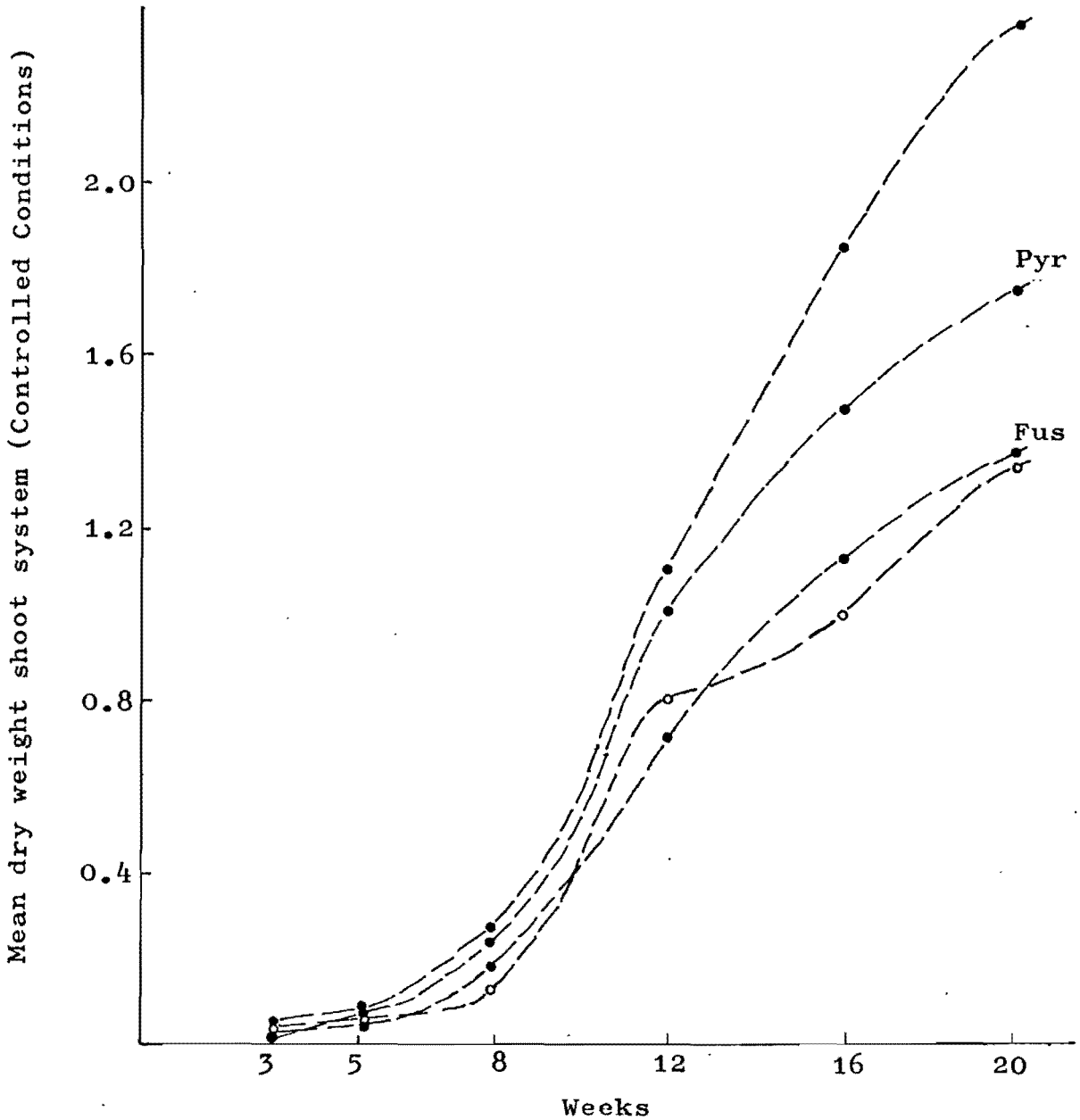


Figure 21. Dry weight of shoot system of onions.

● With Endogone;

○ Without Endogone;

Pyr = With P. terrestris;

Fus = With F. oxysporum;

———Sterilised soil;

Table 17. Statistical Analysis.

Comparing dry weight of plant as affected by Endogone (End.)

in the presence and absence of pathogen under Controlled Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after transplanting	Dry weight shoot system per plant (g)				Analysis of Variance(5%) S...Significant difference I...Insignificant difference			
	+End. (A)	-End. (C)	+F.oxy. +End. (X)	+P.terr. +End. (Y)				
3	0.038	0.030	0.020	0.013	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
5	0.081	0.0071	0.060	0.077	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
8	0.243	0.130	0.189	0.220	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
12	1.118	0.804	0.700	0.992	A Vs X: S;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
16	1.845	1.016	1.125	1.468	A Vs X: S;	C Vs X: I;	A Vs Y: S;	C Vs Y: S;
20	2.373	1.335	1.381	1.752	A Vs X: S;	C Vs X: I;	A Vs Y: S;	C Vs Y: S;

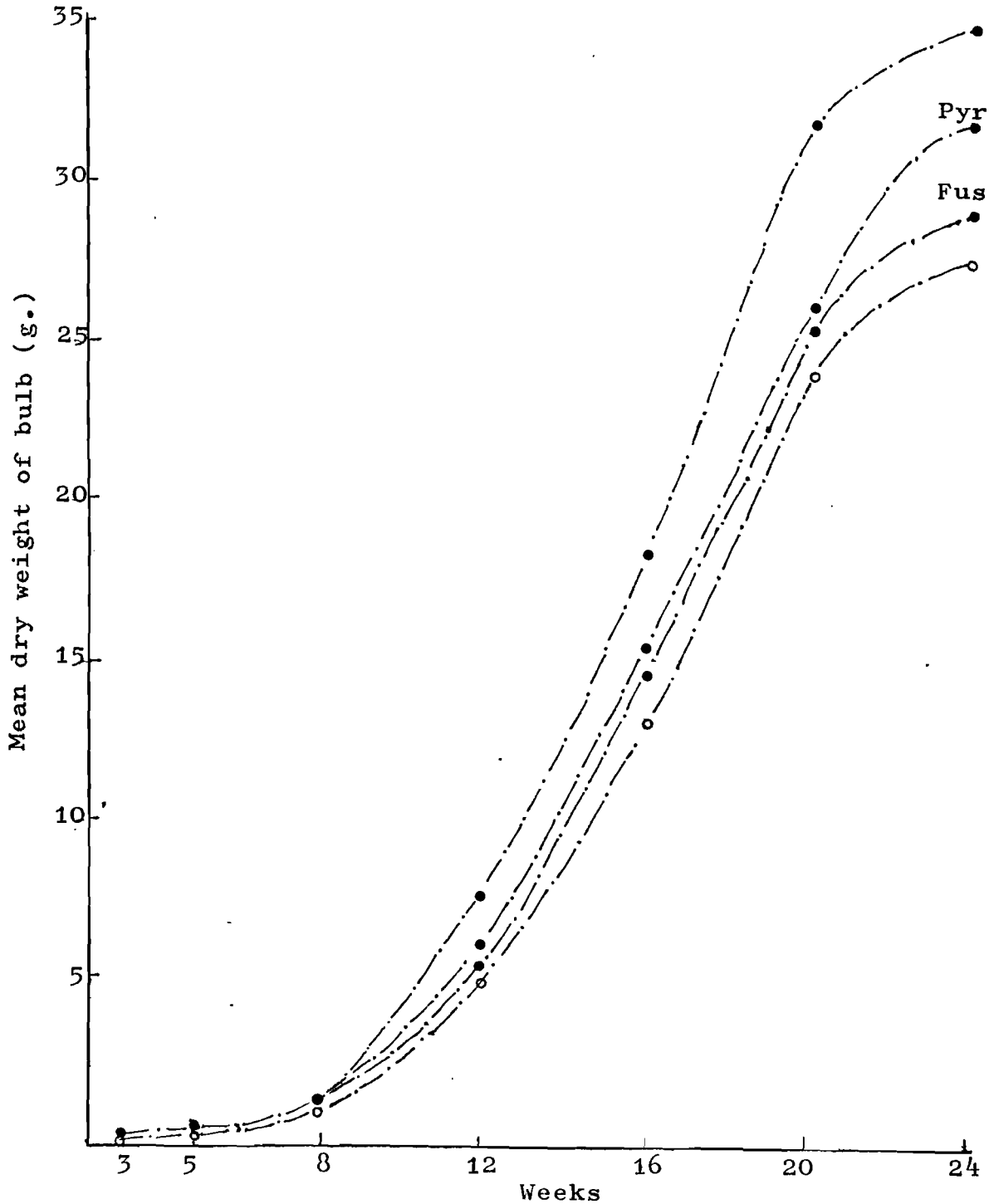


Figure 22. Dry weight of bulb under field conditions.

● With Endogone

○ With Endogone

Pyr = With P. terrestris

Fus = With F. oxysporum

—·—· Unsterilised soil

Table 18. Statistical Analysis.

Comparing dry weight of bulb as affected by Endogone (End.)

in the presence or absence of pathogen-under Field Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after transplanting	Dry weight of bulb per plant(g) +End. (F)	-End. (H)	+F.oxy. +End. (K)	+P.terr. +End. (L)	Analysis of Variance(5%) S...Significant difference I...Insignificant difference
3	0.300	0.185	0.253	0.226	F Vs K: I; H Vs K; I; F Vs L: I; H Vs L: I;
5	0.476	0.318	0.351	0.226	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
8	1.445	1.178	1.509	1.346	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
12	7.950	5.135	5.719	6.069	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
16	18.558	13.430	14.900	15.330	F Vs K: S; H Vs K: I; F Vs L: S; H Vs L: I;
20	32.108	24.488	25.634	26.368	F Vs K: S; H Vs K: I; F Vs L: S; H Vs L: I;
24	35.165	27.818	29.370	32.185	F Vs K: S; H Vs K: S; F Vs L: S; H Vs L: S;

Figure 23. Percentage root colonisation by Endogone (Controlled Conditions).

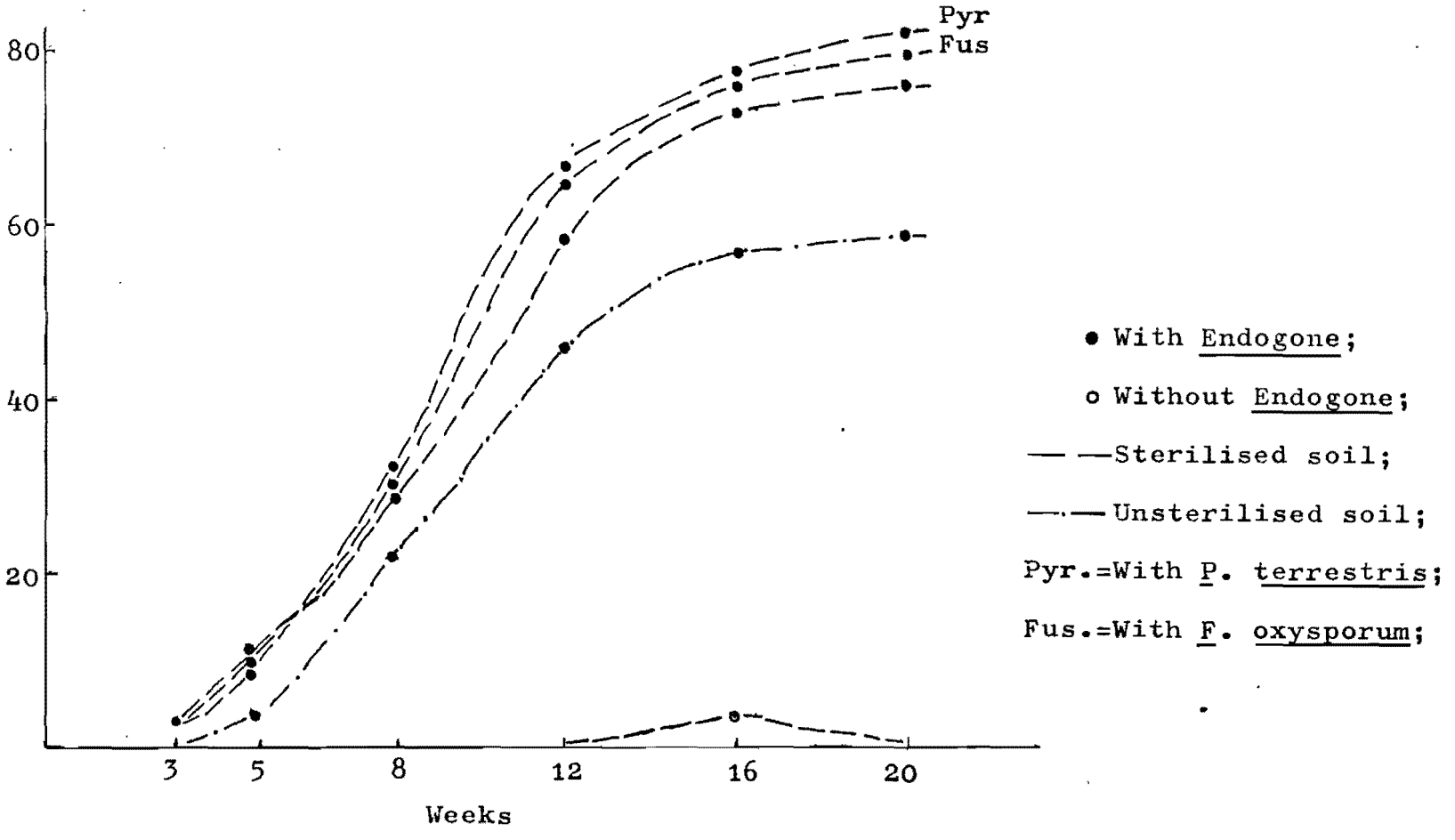


Table 19. Statistical Analysis.

Comparing onion root colonisation by Endogone (End.) in the presence and absence of pathogen under Controlled Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after trans-planting	Mean percentage root colonisation (back-transformed)				Analysis of Variance (5%) S...Significant difference I...Insignificant difference			
	+End. (A)	-End. (C)	+F.oxy. +End. (X)	+P.terr. +End. (Y)				
3	3.000	1.000	2.001	3.000	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
5	11.999	1.000	11.999	10.998	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;
8	29.005	1.000	29.996	31.995	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;
12	59.007	1.000	64.997	67.004	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;
16	73.005	4.001	75.999	77.993	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;
20	75.999	1.000	79.995	82.005	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;

Figure 24. Percentage root colonisation by Endogone (Field Conditions).

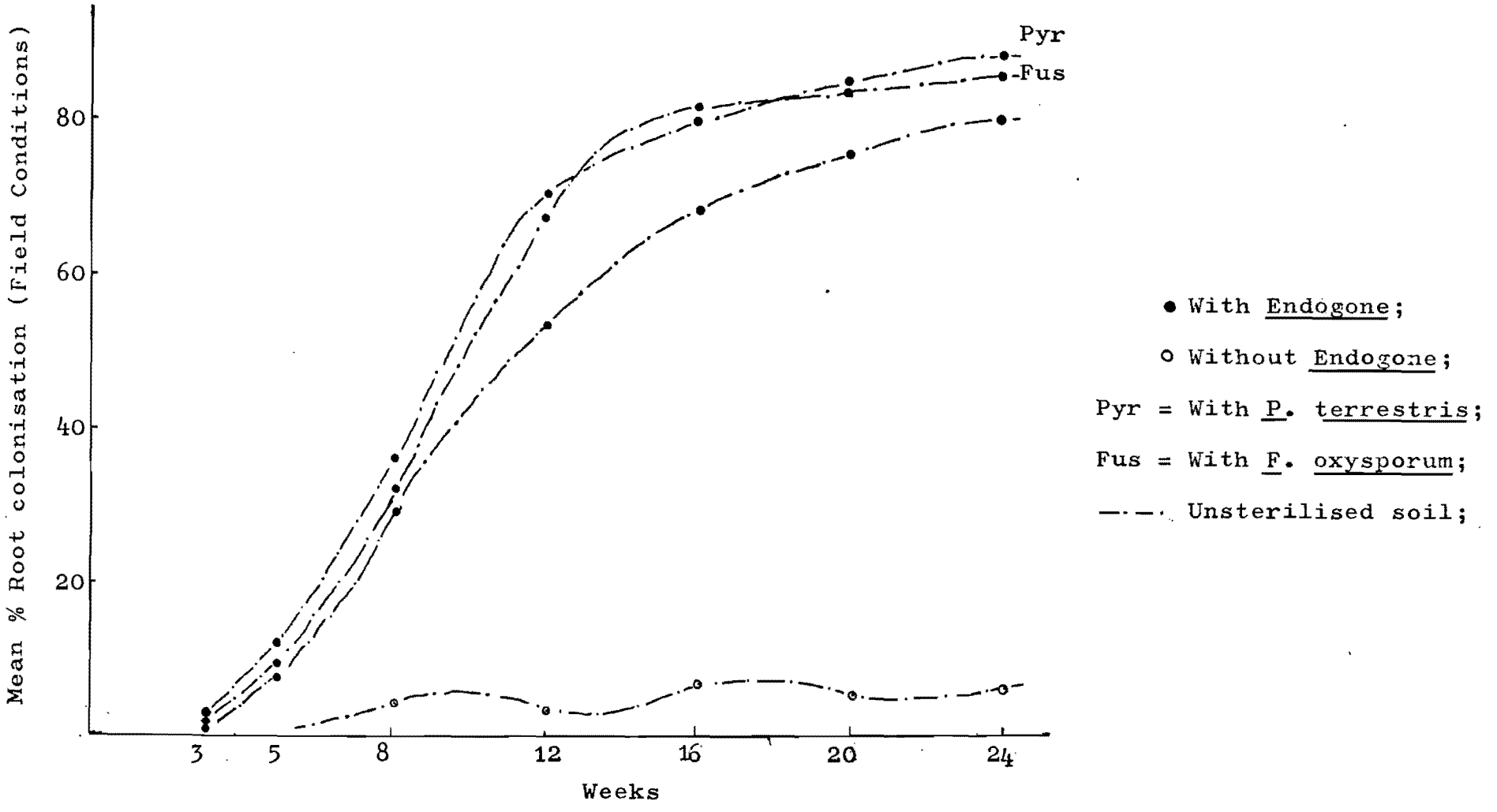


Table 20. Statistical Analysis.

Comparing onion root colonisation by Endogone (End.) in the presence and absence of pathogen under Field Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after trans-planting	Mean percentage root colonisation (back-transformed)				Analysis of Variance (5%) S...Significant difference I...Insignificant difference
	+End. (F)	-End. (H)	+F.oxy. +End. (K)	+P.terr. +End. (L)	
3	1.000	1.000	2.001	3.000	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
5	1.451	1.000	10.998	13.003	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
8	33.001	4.999	33.001	35.004	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;
12	53.995	4.000	70.006	70.993	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;
16	68.992	7.002	80.997	79.995	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;
20	75.999	5.999	84.003	84.993	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;
24	80.997	7.002	86.007	88.992	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;

Figure 25. Dry weight of mycorrhizal roots of onions(Controlled Conditions).

● With Endogone;

o Without Endogone;

Pyr = With P. terrestris;

Fus = With F. oxysporum;

———Sterilised soil;

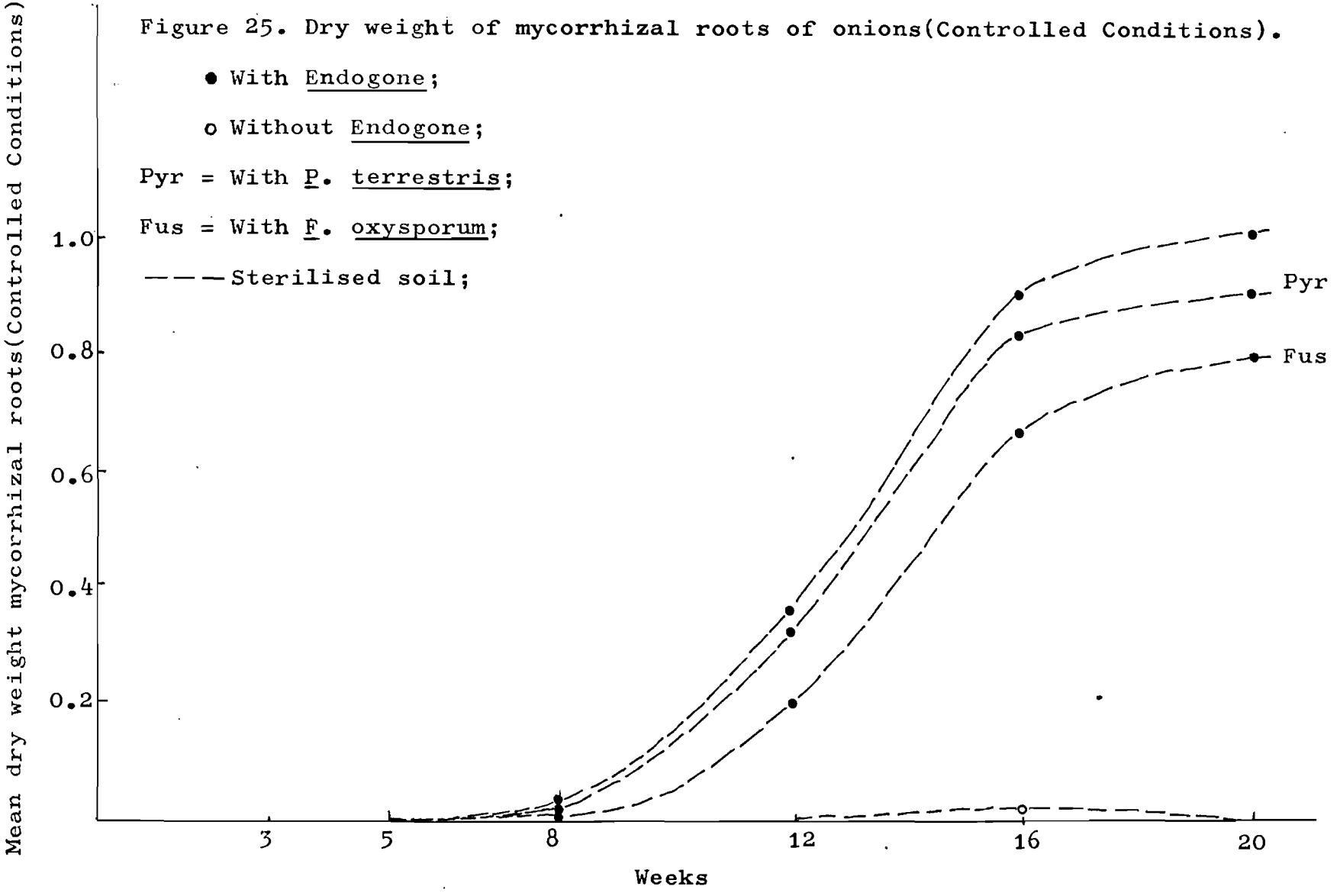


Table 21. Statistical Analysis.

Comparing onion dry weight mycorrhizal roots caused by Endogone (End.) in the presence and absence of pathogen under Controlled Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after transplanting	Mean dry weight mycorrhizal roots (back-transformed)				Analysis of Variance (5%) S...Significant difference I...Insignificant difference			
	+End. (A)	-End. (C)	+F.oxy. +End. (X)	+P.terr. +End. (Y)				
3	1.000	1.000	1.000	1.000	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
5	1.000	1.000	1.000	1.000	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
8	1.031	1.000	1.018	1.026	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
12	1.359	1.000	1.204	1.326	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
16	1.907	1.015	1.666	1.832	A Vs X: I;	C Vs X: I;	A Vs Y: I;	C Vs Y: I;
20	2.045	1.000	1.790	1.890	A Vs X: I;	C Vs X: S;	A Vs Y: I;	C Vs Y: S;

Figure 26. Dry weight of mycorrhizal roots of onions (Field Conditions).

● With Endogone;

o Without Endogone;

Pyr = With P. terrestris;

Fus = With F. oxysporum;

—·— Unsterilised soil;

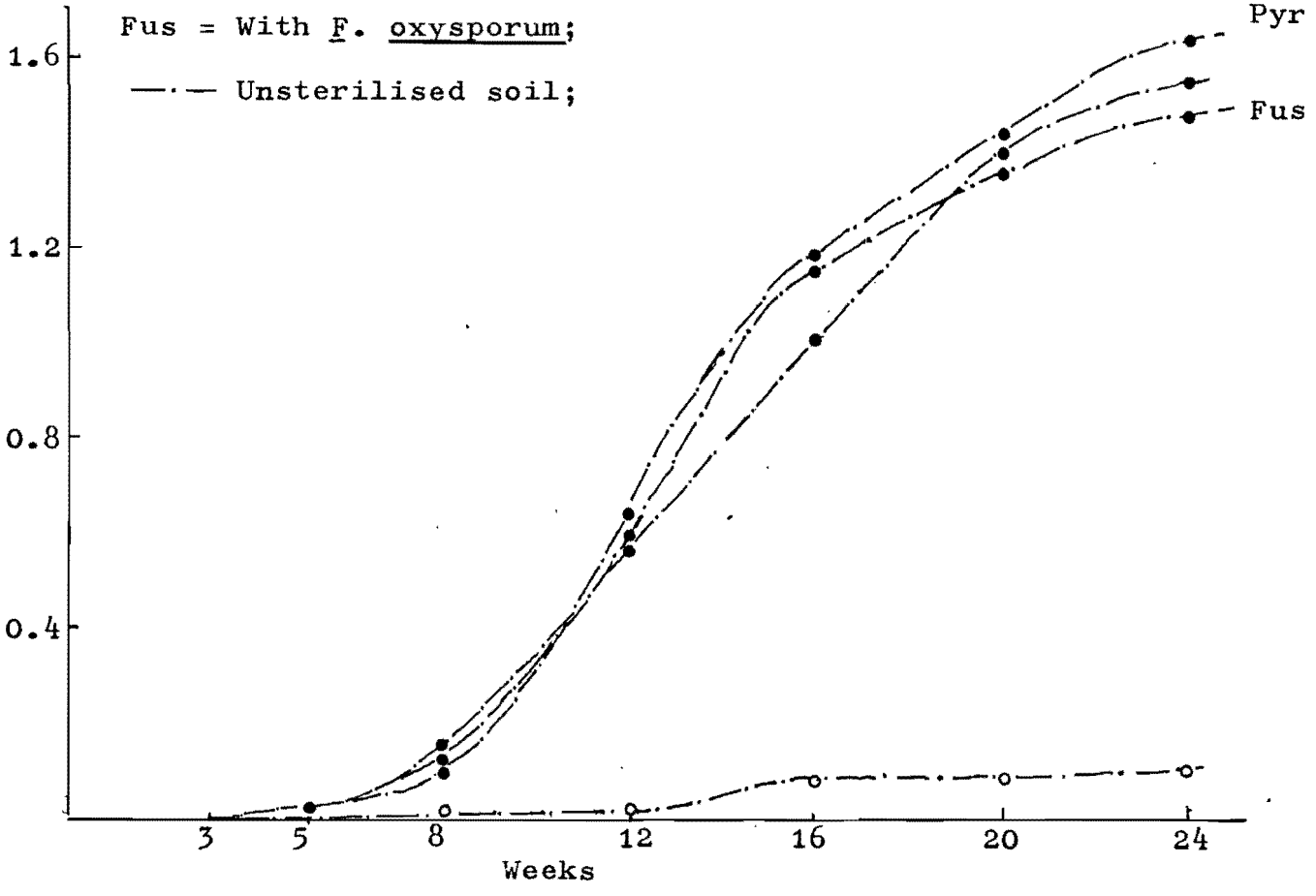


Table 22. Statistical Analysis.

Comparing onion dry weight mycorrhizal roots caused by Endogone (End.) in the presence and absence of pathogen under Field Conditions (5 replicates)

F. oxy. = Fusarium oxysporum; P. terr. = Pyrenochaeta terrestris;

Weeks after trans-planting	Mean dry weight mycorrhizal roots (back-transformed)				Analysis of Variance (5%) S...Significant difference I...Insignificant difference
	+End. (F)	-End. (H)	+F.oxy. +End. (K)	+P.terr. +End. (L)	
3	1.000	1.000	1.000	1.000	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
5	1.020	1.000	1.001	1.003	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
8	1.055	1.014	1.130	1.123	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
12	1.557	1.022	1.571	1.556	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
16	2.095	1.082	2.159	2.185	F Vs K: I; H Vs K: I; F Vs L: I; H Vs L: I;
20	2.407	1.079	2.369	2.429	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;
24	2.542	1.098	2.482	2.640	F Vs K: I; H Vs K: S; F Vs L: I; H Vs L: S;

2. DISCUSSION

The results of this study indicate that plants grown in the presence of *Endogone* show a resistance to *F. oxysporum v. cepa* and *P. terrestris*. This resistance is apparently related to the stage of development of the plant and subsequently to the stage of mycorrhizal development within the root system of the plant.

The observation that young onion plants (3 - 5 weeks old) inoculated with *Endogone* being infected by both the pathogens concerned may be attributed to a low or nil establishment of mycorrhiza within the root system of the host, as shown by Figure 2, where mycorrhizal development was estimated to be only about 10%. A definite reduction in the proportion of plants infected by disease was seen in the 12th week (Figures 17 and 18) when mycorrhizal development was well established. At the end of the experiment, only 52 - 57% of *Endogone* treated plants were infected by the two pathogens, the lowest incidence of disease during the period of maximum mycorrhizae development.

It may be thus proposed that the mycorrhizal plants had been afforded some means of protection against *F. oxysporum v. cepa* and *P. terrestris*. The mechanism or mechanisms by which protection was achieved may be speculated upon. *Endogone* is thought to occupy more host tissue than all the other fungal parasites put together (Gerdemann, 1968). As such, *Endogone* mycorrhiza may be a physical barrier to

the pathogens concerned. Wilhelm (1959) once thought Endogone to be harmful to strawberries, because colonisation was so abundant in the second year that rootlets were ruptured by it. Such a successful coloniser to which the host is not hypersensitive may act as a "territorial claimant" and the tissues occupied by them may tend to be inhospitable to invasion by other fungi. The juvenile susceptibility of the Endogone treated plants to the two pathogens may be attributed to the young roots not being territorially occupied. Wilhelm (1973) also discussed the possibility of root diseases in old plants caused by pathogens of the genera Fusarium, Verticillium and Phytophthora, which invade near the root tip, to reflect the fact that root tips are typically not occupied by Endogone.

Another possible mechanism of antagonism by which Endogone may have suppressed F. oxysporum v. cepa and P. terrestris may have been by affecting root exudates from Endogone treated plants. Exudates from mycorrhizal roots probably differ from those of non-mycorrhizal roots due to the nutritional status of the mycorrhizal plant being altered and also because the large volume of fungus tissue in mycorrhizal roots may affect the rhizosphere microorganisms and hence render mycorrhizal roots less susceptible to invasion by other fungi. Davey (1971) and Marx (1971) have reported of such antagonistic effects. The presence of antibiotics have been detected by some workers from root

extracts of *Allium* plants. (Cavallito, Buck and Suter, 1944; Clarke, 1966; Hayes, 1946; Virtanen and Matikkala, 1959; Yoshimura, Tsuno and Marakami, 1958). The presence of *Endogone* may have induced the production of antibiotics which were inhibiting to the pathogens. *Endogone* itself may have been involved in some form of antibiosis whereby it produces toxic metabolites as an adaptive phenomenon in response to the presence of the pathogens.

The results obtained in this experiment did not lead to the actual mechanism that may have been involved in the reduction of the disease symptoms of *F. oxysporum v. cepa* and *P. terrestris*. There may even have been a more complex combination of mechanisms at work.

CHAPTER VII

TESTING THE IMPORTANCE OF PHOSPHATE NUTRITION ON PLANT GROWTH AND DISEASE RESISTANCE

1. RESULTS

1.1. Effect of phosphate applications on phosphate uptake of onions.

At the 0.1 g. $CaHPO_4 . 2H_2O$/kg. soil application level (Table 23), Endogone treated plants were found to contain about twice (0.32%) the amount of phosphorus than that found in the control plants (0.15%). At the 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil level, the lead was further increased. But at the 1.0 g. $CaHPO_4 . 2H_2O$/kg. soil application level, Endogone treated plants showed a decrease in phosphorus content while the control plants showed a slight increase. At the 2.0 g. $CaHPO_4 . 2H_2O$/kg. soil level, both Endogone treated and control plants showed increases in phosphorus content (Figure 28).

1.2. Effect of phosphate applications on v.a. mycorrhizal development.

Root colonisation by Endogone was fairly high (70%) with the addition of 0.1 g. $CaHPO_4 . 2H_2O$/kg. soil and this increased to 85% at the 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil application level (Figure 27). With the addition of 1.0 g. $CaHPO_4 . 2H_2O$/kg. soil, root colonisation dropped drastically to 10% and at the 2.0 g. $CaHPO_4 . 2H_2O$/kg. soil application level, root colonisation was non-existent. Control plants did not show any mycorrhizal development

(Table 23).

A known quantity of the soil which received 2.0 g. $CaHPO_4 . 2H_2O$/kg. soil was added to a similar quantity of fresh sterilised soil (a 1:1 "dilution") to reduce the amount of phosphate present to approximately 1.0 g. $CaHPO_4 . 2H_2O$/kg. soil and used to grow other control plants. These plants showed between 20 - 30% colonisation.

1.3. Effect of phosphate applications on onion shoot and root growth.

Shoot and root growth increased with the addition of 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil and started to level off with 1.0 g. $CaHPO_4 . 2H_2O$/kg. soil (Figure 28). Endogone treated plants demonstrated greater shoot and root growth than the controls. The addition of 2.0 g. $CaHPO_4 . 2H_2O$/kg. soil caused a decrease in shoot and root growth.

1.4. Effect of phosphate application on disease resistance of onions.

At the 0.1 g. and 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil application levels, the number of Endogone treated plants which became infected by the pathogens was fairly low, that is, between 56 - 62% (Figure 27). This was more so at the 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil application level, especially with regard to P. terrestris, where only 53% of Endogone treated plants became diseased (Table 23). At the 1.0 and 2.0 g. $CaHPO_4 . 2H_2O$/kg. soil levels, disease infection was high (72 - 80%) in Endogone treated plants. Control plants became heavily infected with both pathogens at all levels of $CaHPO_4 . 2H_2O$ application.

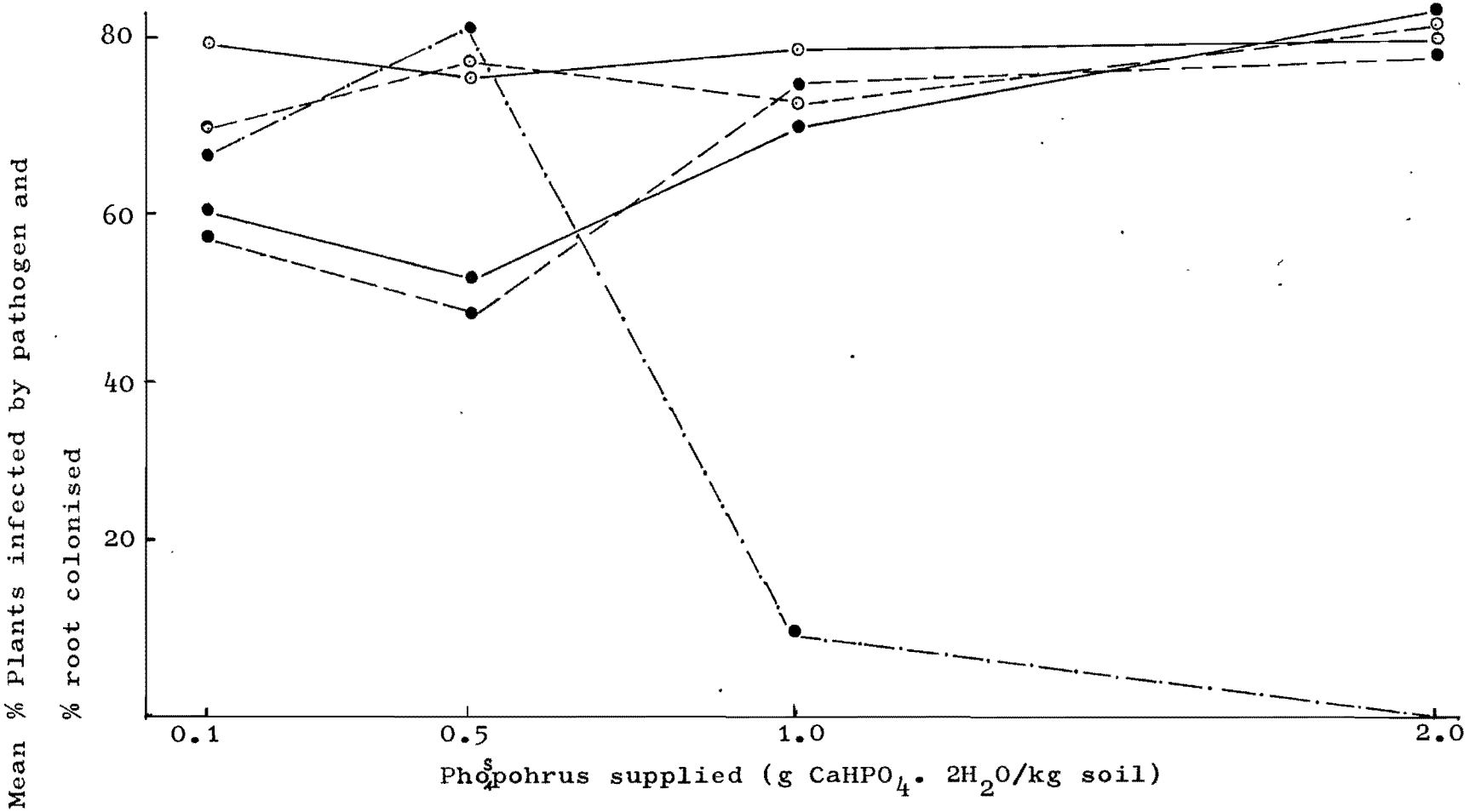


Figure 27. % of onions infected by pathogens and % onion root colonisation by Endogone.

● With Endogone; ○ Without Endogone;

---- With P. terrestris; ——— With F. oxysporum;

—·— % root colonisation

Figure 28. Shoot and root growth rates and phosphate content of onions.

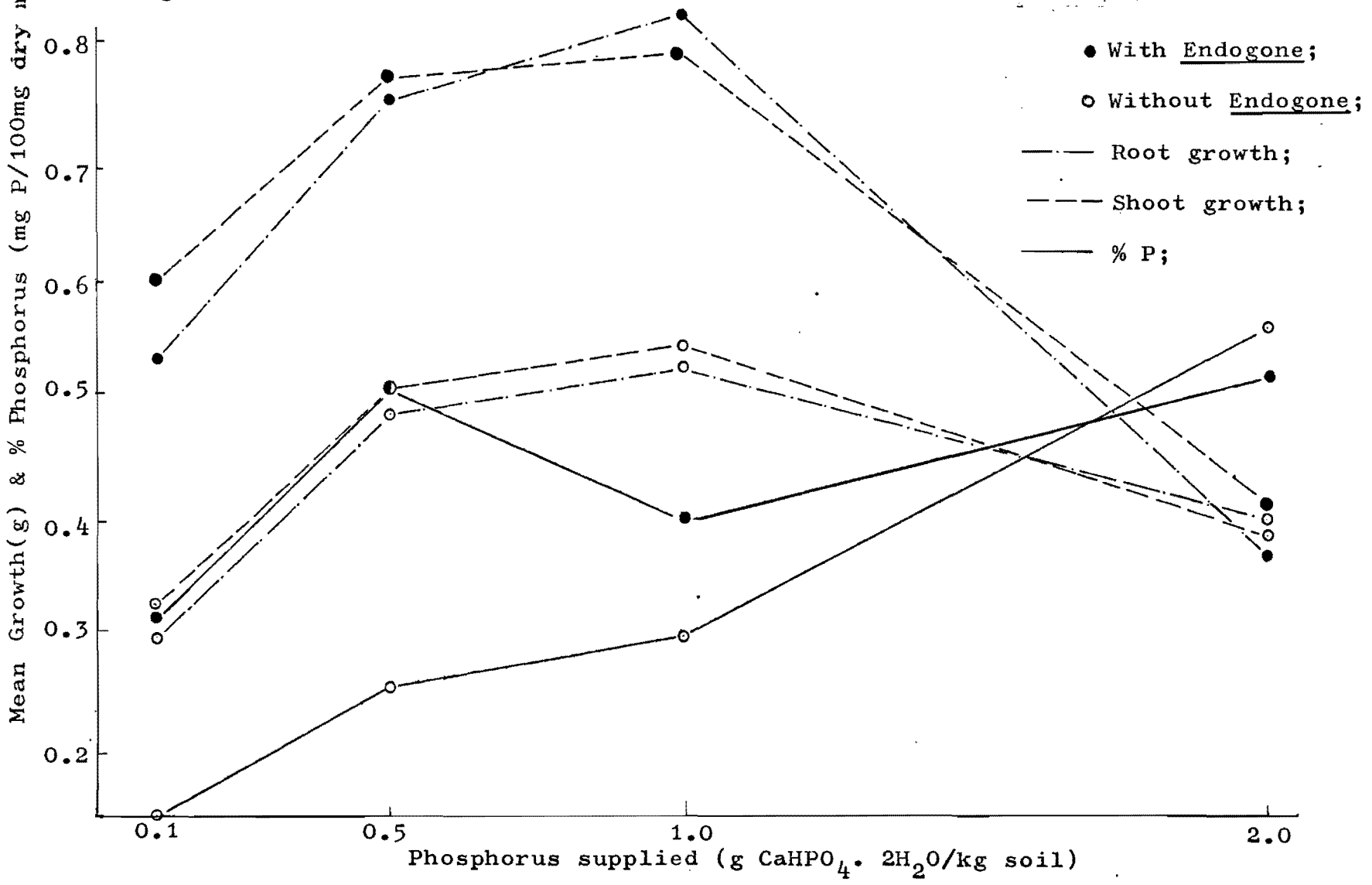


Table 23. Importance of phosphate ($CaHPO_4.2H_2O$) nutrition on plant growth and disease resistance of twelve-week old onions (5 replicates). End. = Endogone

$CaHPO_4.2H_2O$ supplied	0.1 g/kg soil		0.5 g/kg soil		1.0 g/kg soil		2.0 g/kg soil	
Endogone treatment	+End. (A)	-End. (B)	+End. (C)	-End. (D)	+End. (E)	-End. (F)	+End. (G)	-End. (H)
Root weight (g)	0.555	0.300_S	0.757	0.477_S	0.810	0.533_S	0.381	0.435_I
Shoot weight (g)	0.662	0.359_S	0.781	0.493_S	0.793	0.529_S	0.427	0.400_I
% root colonisation by Endogone	70.20	0.00_S	85.40	0.00_S	10.20	0.00_I	0.00	0.00_I
% phosphorus (mg P per 100mg dry matter)	0.318	0.156_S	0.511	0.256_S	0.417	0.359_I	0.519	0.577_I
% F. oxysporum infection	62	80_S	56	76_S	72	82_I	80	80_I
% P. terrestris infection	58	72_S	53	82_S	76	74_I	80	88_I

Note: The letters 'S' and 'I' in the B, D, F, and H cells refer to the Analysis of Variance tests on the differences between A & B, C & D, E & F, and G & H, respectively, being significant or insignificant (5% level).

2. DISCUSSION

Inspection of the results (Figure 28) shows that the synthesis of v.a. mycorrhiza in culture is much affected by amounts of phosphate in the media. Optimum amounts of $CaHPO_4 . 2H_2O$ were of the order of 0.5 g./kg. of soil (Figure 28). Mycorrhizal development was diminished when an extra 0.5 g. $CaHPO_4 . 2H_2O$/kg. soil was supplied. Mosse and Hayman (1971) reported similar results of optimum phosphorus concentration for v.a. mycorrhiza in Trifolium parviflorum. This adverse effect on mycorrhizal development is more obvious with the complete suppression of v.a. mycorrhizae in plants supplied with 2.0 g. $CaHPO_4 . 2H_2O$.

The tendancy for mycorrhizal infection to diminish when much phosphate has been supplied has been reported in soils (Baylis, 1967; Mosse, 1967), in sand culture (Daft and Nicolson, 1969) and in agar media containing more than 30 ppm soluble phosphorus (Mosse and Phillips, 1971). Mosse (1973) proposed that in the presence of high levels of phosphate, most cortical cells in the onion root become resistant to Endogone infection, rather than the Endogone inoculum being killed by large doses of phosphate. The soil dilution part of this experiment confirms the latter.

The phosphorus content of plants was seen to increase steadily with increasing levels of phosphate supplied. The decrease in phosphorus content in Endogone treated plants from 0.51% at the 0.5 g. $CaHPO_4 . 2H_2O$ level to 0.4% at the 1.0 g. $CaHPO_4 . 2H_2O$ level may be attributed to the different

extent of mycorrhizal development at the two levels. At the lower level, the proliferation of mycorrhiza synthesis may have increased phosphorus content in the plant. At the higher level, the reduction of mycorrhizal development may have led to a slight decreased phosphate uptake, which, nevertheless, was greater than that in control plants (0.30%).

The phosphorus content for optimum plant growth was between 0.3 - 0.4%. Higher phosphorus content brought about reduced plant growth (Figure 27). Apparently, phosphorus concentration in plants can reach levels which are detrimental to plant growth. This in agreement with the findings of Mosse (1972). The condition of phosphate toxicity is probably less readily reached in clay containing soils where much of the added phosphate rapidly becomes unavailable to plants, than in light or sandy soils, as was used in this experiment, where the added phosphate remains available.

Phosphate toxicity has been reported by numerous other workers, especially in pot grown plants such as oats (Rossiter, 1952, 1955); wheat (Bhatti and Loneragan, 1970); lupins (Warren and Benzian, 1959); subterranean clover (Greenwood and Hallsworth, 1960); soyabean varieties (Foote and Howell, 1964) and onions (Mosse, 1973). It has also been described under field conditions, among others by Benzian, Freeman and Patterson (1972) in Sitka spruce, Rossiter (1955) in subterranean clover, Ross (1971) in soyabeans and Khan (1972, 1973) in wheat. Different species

of plants differ in the level of phosphorus at which it becomes toxic. Toxicity in onions apparently develop at tissue concentrations above 0.4% phosphorus in this study, while most other plants mentioned here do not reach such supra-optimal levels below 1.0% phosphorus. One method of counteracting the effects of too much phosphorus is to starve the plants of nitrogen, (Mosse, 1962; Baylis, 1967; Mosse, 1967) thereby restricting the energy required for phosphorus absorption. Conversely, phosphate toxicity may be reduced by adding nitrogen (Greenwood and Hallsworth, 1960; Bhatti and Loneragan, 1970) whereby growth is encouraged, hence decreasing plant phosphorus concentration.

The percentage of Endogone treated plants that was infected with the two pathogens was lowest (53 - 62%) when 0.1 and 0.5 g. $CaHPO_4 \cdot 2H_2O$/kg soil were applied, more so at the 0.5 g. $CaHPO_4 \cdot 2H_2O$ level. This was significantly lower than the percentage of the control plants (72 - 82%) that were infected with the two pathogens. At the 1.0 and 2.0 g. $CaHPO_4 \cdot 2H_2O$/kg. soil levels, both Endogone treated and control plants became heavily infected by the two pathogens. This difference in susceptibility may be attributed to the degree of mycorrhizal development. The lower levels of phosphate application which resulted in a lower susceptibility to the diseases had also been shown to favour mycorrhizal development. It may be postulated then that the abundance of mycorrhizae is related to a reduction in disease infection. Thus, the higher phosphate application levels which would have suppressed mycorrhizal development may have removed one possible means of

protection against F. oxysporum v. cepa and P. terrestris.

These results indicate that the mere presence of phosphorus alone either through absorption by Endogone or artifically applied is probably not the major factor contributing to a lower susceptibility to the two pathogens. Thus, the presence of v.a. mycorrhizae may offer not only a means of increasing phosphorus uptake but also play a protective role against soil borne pathogens such as F. oxysporum v. cepa and P. terrestris.

CHAPTER VIII

CULTURING V.A. MYCORRHIZA IN *ALLIUM CEPA* IN AGAR MEDIA

1. RESULTS

1.1. Effect of phosphate source on root colonisation

Plants supplied with calcium phytate (Figure 29, media 4 and 9) as the main source of phosphorus demonstrated the highest root colonisation. Those supplied with $CaHPO_4 \cdot 2H_2O$ (Figure 20, media 2 and 7) showed next highest root colonisation, followed by those given inositol (Figure 29, media 3 and 8).

Plants which received sodium phytate as the phosphorus source (Figure 29, media 5 and 10) showed the lowest colonisation, being lower than those given inositol, and those not supplied with phosphate at all (Figure 29, media 1 and 6).

1.2. Effect of phosphate source on shoot and root growth of onions

The trend of shoot growth was very similar to that of percentage root colonisation with the greatest rates being shown by plants supplied with calcium phytate and the lowest by those given sodium phytate (Figure 32). Root growth exhibited similar records, with one exception, that is, plants grown with $FeCl_3$ but no phosphate (Figure 31, medium 1) showed the least root growth instead of those

given sodium phytate.

1.3. Effect of phosphate source on percentage phosphorus dry matter

Highest percentage phosphorus dry matter were recorded of plants grown in calcium phytate, followed by those given $CaHPO_4 . 2H_2O$ (Figure 30). Sodium phytate given plants followed next, being slightly greater than those supplied with inositol. Plants not supplied with phosphate at all resulted in the lowest percentage phosphorus dry matter.

1.4. Comparing effects of $FeCl_3$ and Fe K EDTA (Ferric potassium ethylenediaminetetra acetic acid)

Generally, plants grown in all media provided with Fe K EDTA exhibited greater root colonisation, shoot weight, root weight and percentage phosphorus dry matter (Table 24). However, four (out of twenty) exceptions occured, namely in the root colonisation of plants provided with inositol (Figure 29, media 3 and 8), in the shoot weight (Figure 32, media 5 and 10) and root weight (Figure 31, media 5 and 10) of plants provided with sodium phytate and in the percentage phosphorus dry matter of plants supplied with $CaHPO_4 . 2H_2O$ (Figure 30, media 2 and 7).

Table 24. Effect of various phosphate sources on the establishment of vesicular arbuscular mycorrhiza (5 replicates)

Medium number	Main source of phosphate	Phosphate supplied mg P/l	Mean % Root colonised	Mean Shoot weight (g)	Mean Root weight (g)	Mean % P (mg P per 100mg dry matter)
1	None	0	43	0.281	0.201	0.050
2	$CaHPO_4 . 2H_2O$	100	74_S	0.389_I	0.322_I	0.274_S
3	None	0	63_S	0.333_I	0.270_I	0.154_I
4	Ca phytate	100	85_S	0.459_S	0.372_I	0.308_S
5	Na phytate	100	30_S	0.255_I	0.234_I	0.203_I
6	None	0	51	0.301	0.252	0.118
7	$CaHPO_4 . 2H_2O$	100	82_s	0.417_i	0.351_i	0.244_i
8	None	0	56_i	0.351_i	0.297_i	0.188_i
9	Ca phytate	100	94_s	0.503_s	0.418_i	0.347_i
10	Na phytate	100	37_s	0.233_i	0.218_i	0.248_i

Note: Media 1 - 5 were supplied with $FeCl_3$ and media 6 - 10 with Fe K EDTA
Media 3 & 8 were supplied with Inositol in addition.
S, I and s, i = Significant or insignificant differences (ANOVA) between media 1 and 2, 3, 4 or 5 and between media 6 and 7, 8, 9 or 10, respectively (5%)

Figure 29. % root colonisation by Endogone.

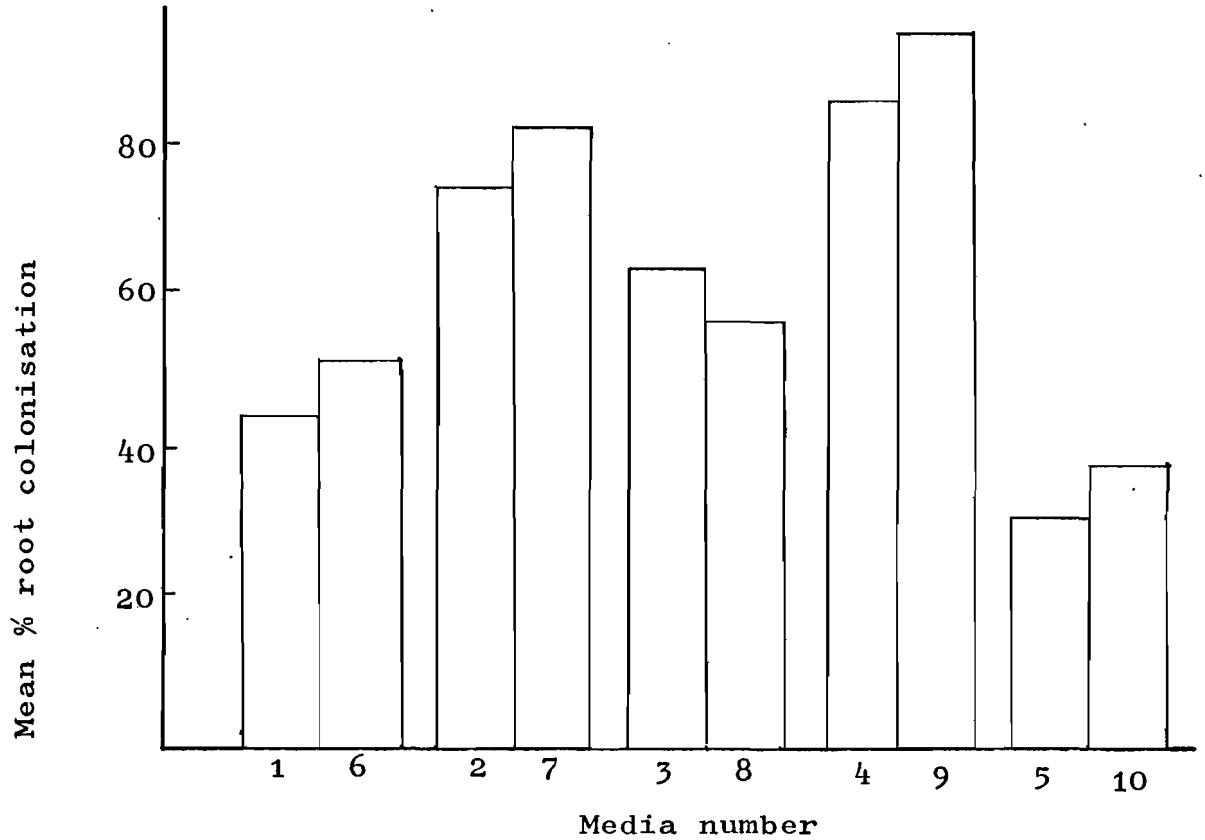


Figure 30. Phosphorus (%) content of onions.

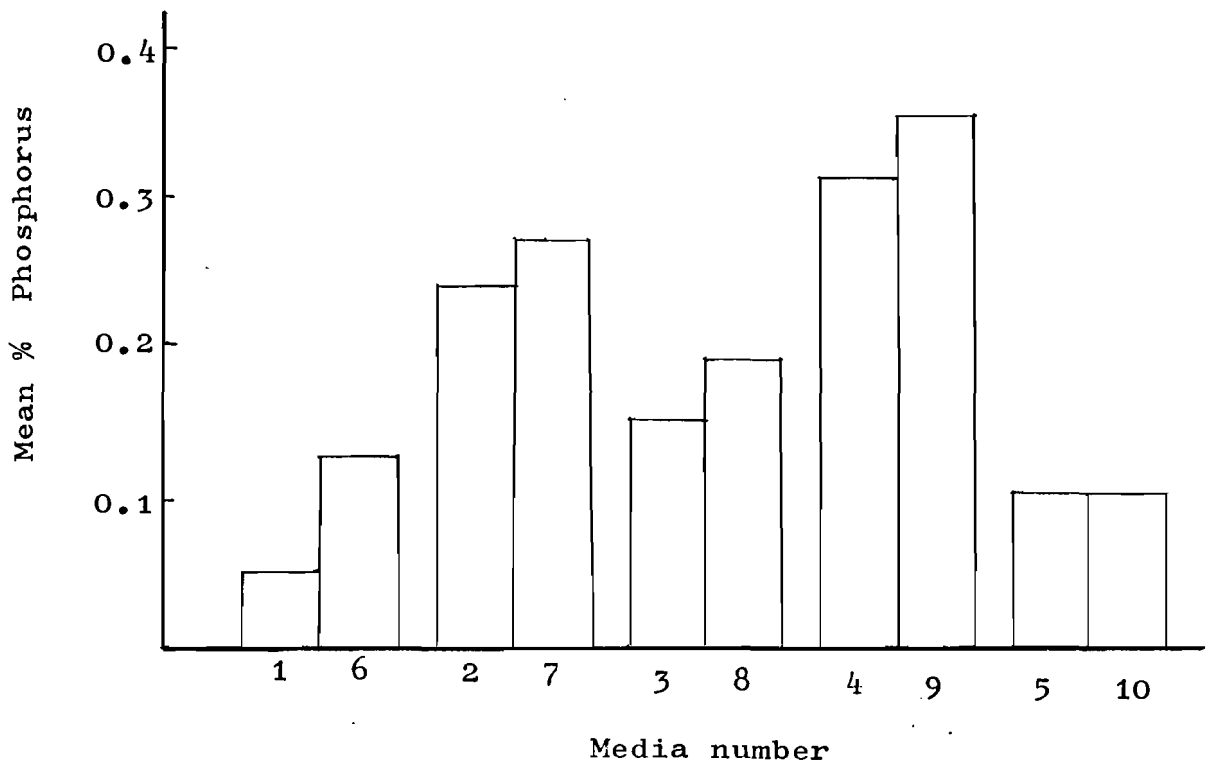


Figure 31. Dry weight of root system of onions

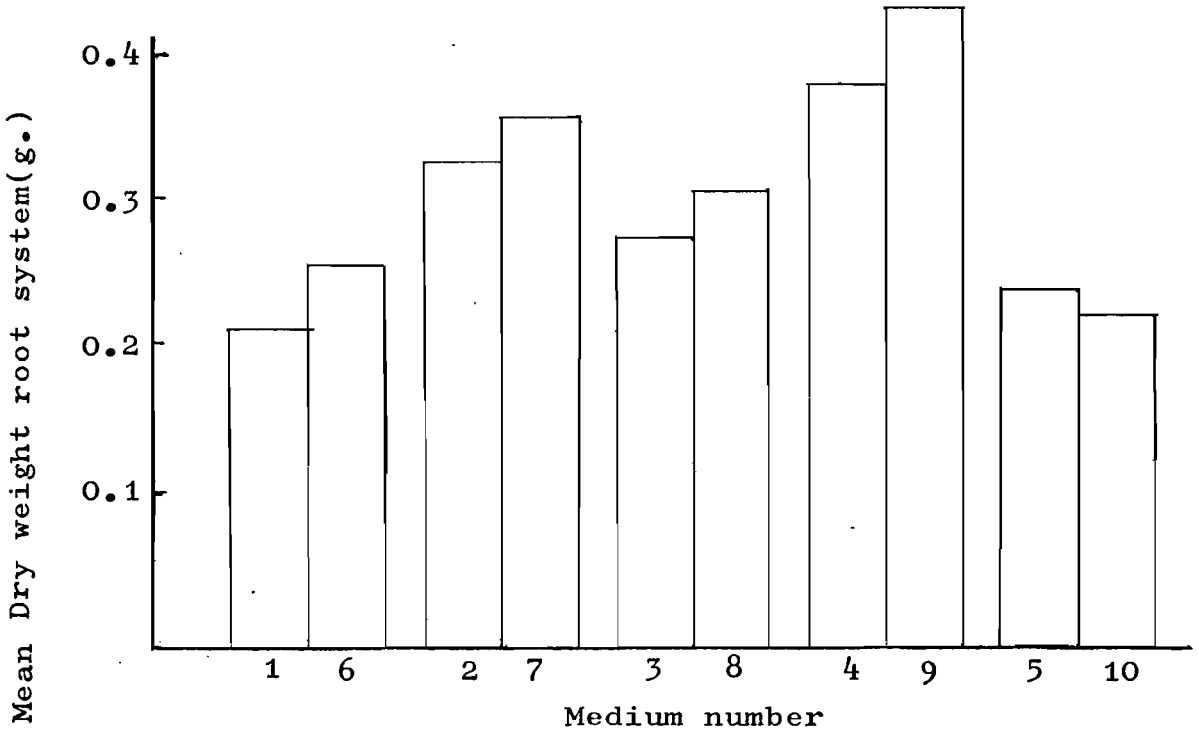
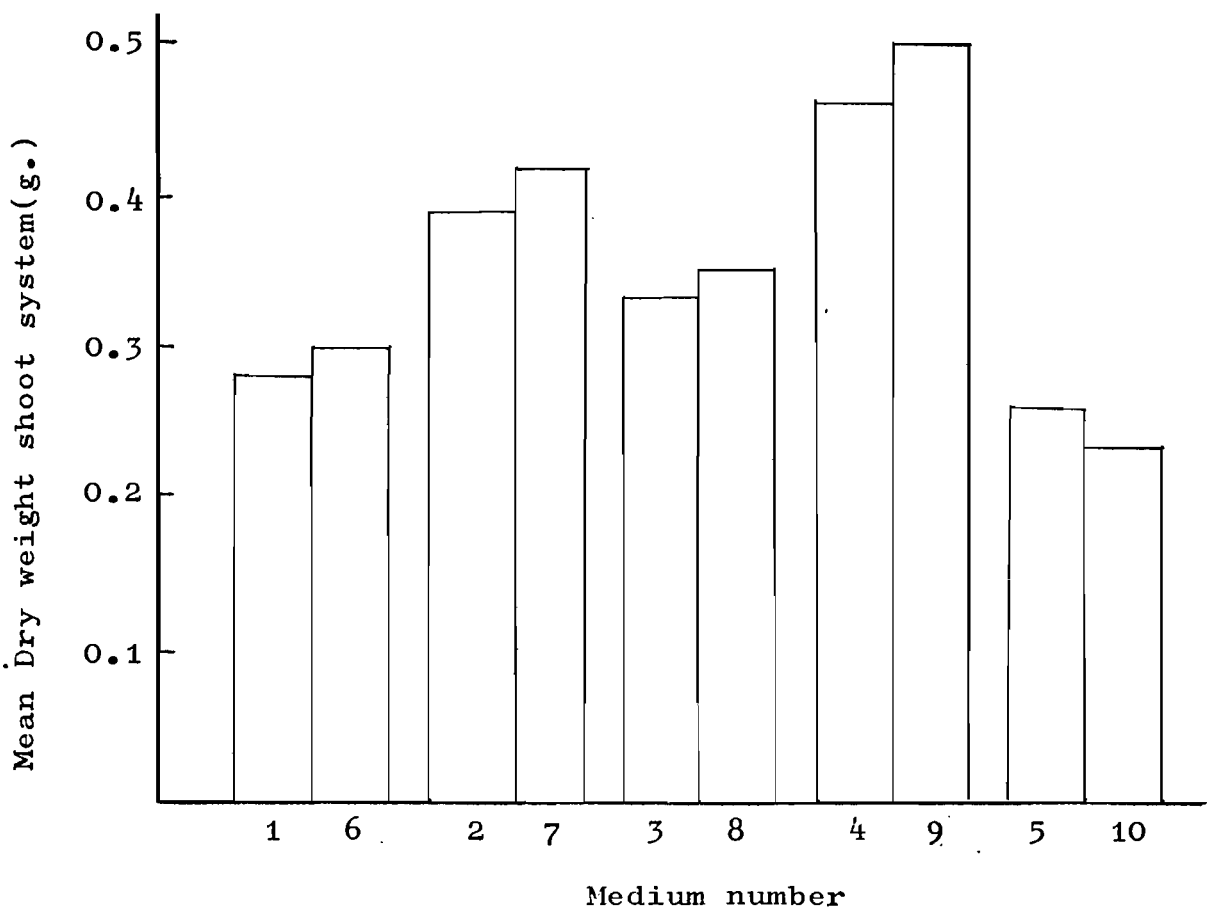


Figure 32. Dry weight of shoot system of onions



2. DISCUSSION

The observations gave a fairly clear indication as to the optimum phosphorus requirements for the growth of onions and the development of v.a. mycorrhizae, the amount for the latter being considerably lower (0.5 g. $CaHPO_4 \cdot 2H_2O$/ kg. soil) than the former (1.0 g. $CaHPO_4 \cdot 2H_2O$/kg. soil). The amount of phosphorus in the media used in this study had been regulated to best encourage the mycorrhizal infections and yet be sufficient for the growth of onions. The main aim in this study was culturing the v.a. fungi rather than promoting the growth of the onions.

The results show that of the forms of phosphate supplied to the plants, calcium phytate elicited the greatest response in mycorrhizal development and plant growth. Nevertheless, all other forms of phosphate stimulated mycorrhizal development and plant growth, indicating that the former and latter are generally independent of type of phosphate supplied.

Inositol appeared to enhance mycorrhizal development which in turn may have been responsible for the relatively high growth rates (third highest), despite the fact that inositol is phosphorus free. This may be attributed to inositol functioning as a carbon source for the fungi, which in the inorganic medium may have been the only carbon source available, apart from possible contributions on the part of the plant, or more unlikely, from atmospheric CO_2 (Mosse and Phillips, 1971). Also the amount of inositol supplied (0.12 g/l) is that which is

present in 0.63 g. calcium phytate. Hence, part of the enhanced mycorrhizal development and plant growth seen with calcium phytate may have been due to its inositol component acting as a carbon source. Anderson (1956) has shown that the major part of the organic phosphate fraction in soils consists of phytates. Phytates are not readily attacked by most micro-organisms, thus offering a readily available source of carbon to Endogone.

The sodium salt of phytic acid, however, unlike its calcium counterpart, seem to have an adverse effect on mycorrhizal development and plant growth. The sodium content or part is probably the cause of the suppression, though the exact cause is not clear (Mosse and Phillips, 1971; Mosse and Hepper, 1975). Mosse and Phillips (1971) also suggested that the high sodium content in Jensen's (1942) medium may have contributed to the difficulties previous workers encountered in attempting to obtain v.a. mycorrhizae in Jensen's medium. In addition, Jensen's medium also has a high phosphorus content (265 mg/l) which would have probably caused the phosphate uptake by the plants under study to reach supra-optimal levels. The adverse phosphate toxicity effects at these levels may have affected the plants susceptibility to Endogone infection. Also, such high phosphate levels may have adversely affected the fungus itself.

Varying the iron source in the medium did not seem to affect the development of v.a. mycorrhiza appreciably. Statistical tests (analysis of variance) showed no

significant effect in varying the iron source. However, the use of Fe K EDTA generally improved root colonisation by Endogone and plant growth.

These observations indicate that ungerminated Endogone spores can be used to obtain typical v.a. infections in onions, thus dispensing with the need to use spores previously germinated, the procedure of which is time consuming, as shown by Mosse (1962). Similar results have been reported by Mosse and Phillips (1971). They established v.a. mycorrhiza with ungerminated Endogone spores in Trifolium parviflorum.

Pearson and Read (1973) described the biology of ericaceous mycorrhiza in which they isolated the endophyte from various sources, namely from ericaceous roots and soil fragments and then resynthesized mycorrhizae with the isolates obtained, however, they failed to clearly mention the form in which the isolates were introduced as inoculum.

CHAPTER IX

GERMINATION OF ENDOGONE SPORES

1. RESULTS

All media showed greater germination rates than medium 1 (Table 25). Among the media that received $CaHPO_4.2H_2O$, calcium phytate and inositol, media that received calcium phytate exhibited the highest germination rates, followed by those which received $CaHPO_4.2H_2O$ and then by those which received inositol. There were two exceptions, namely, spore source 100 in media 2, 3 and 4, placed in the wooden incubator and spore source 200 in media 10, 11 and 12, placed in the metal incubator, where the calcium phytate receipients showed the lowest germination rates.

The addition of $CaHPO_4.2H_2O$, calcium phytate and inositol to water agar improved germination rates considerably, except for that of spore source 100 placed in the wooden incubator, where all three additions resulted in decrease in germination.

The addition of nicotinic acid and thiamine HCL separately to water agar further increased germination percentage. The separate further addition of nicotinic acid (Media 7, 8 and 9) and thiamine HCl (Media 10, 11 and 12) to $CaHPO_4.2H_2O$, calcium phytate and inositol showed even greater increased germination rates, with those receiving nicotinic acid being superior.

The simultaneous addition of both nicotinic acid and thiamine HCl to water agar produced even greater a germination rate. The simultaneous addition of both nicotinic acid and thiamine HCl to $CaHPO_4.2H_2O$ (Medium 13), calcium phytate (Medium 14) and inositol (Medium 15) demonstrated the greatest germination rates recorded.

It was also observed that spores from both sources generally germinated better when placed in the wooden incubator (Figures 33 and 34), except for those germinated in media 1 and 12 of spore source 200 (i. e. 2 out of 16) and in media 3, 9 and 15 of spore source 100 (i. e. 3 out of 16).

Of the two spore types used, those of source 200 exhibited greater germination percentages in most media and in both incubators (Figures 35 and 36) except for those germinated in media 4, 11, 13 and 14 in the metal incubator (1. e. 4 out of 16) and media 1 and 12 in the wooden incubator (i. e. 2 out of 16).

Table 25. Germination of Endogone spores.

	Mean Percentage germination(5 replicates)			
	In metal incubator		In wooden incubator	
Medium	Spore Source 100	Spore Source 200	Spore Source 100	Spore Source 200
1	48	65	69	54
2	60	68	67	72
3	64	70	59	76
4	53	39	60	50
5	69	76	72	80
6	66	70	78	82
7	70	76	75	81
8	78	80	84	88
9	70	73	64	77
10	66	70	70	75
11	69	62	76	70
12	68	70	72	65
13	80	78	83	85
14	85	82	88	90
15	76	80	70	85
16	73	76	75	80

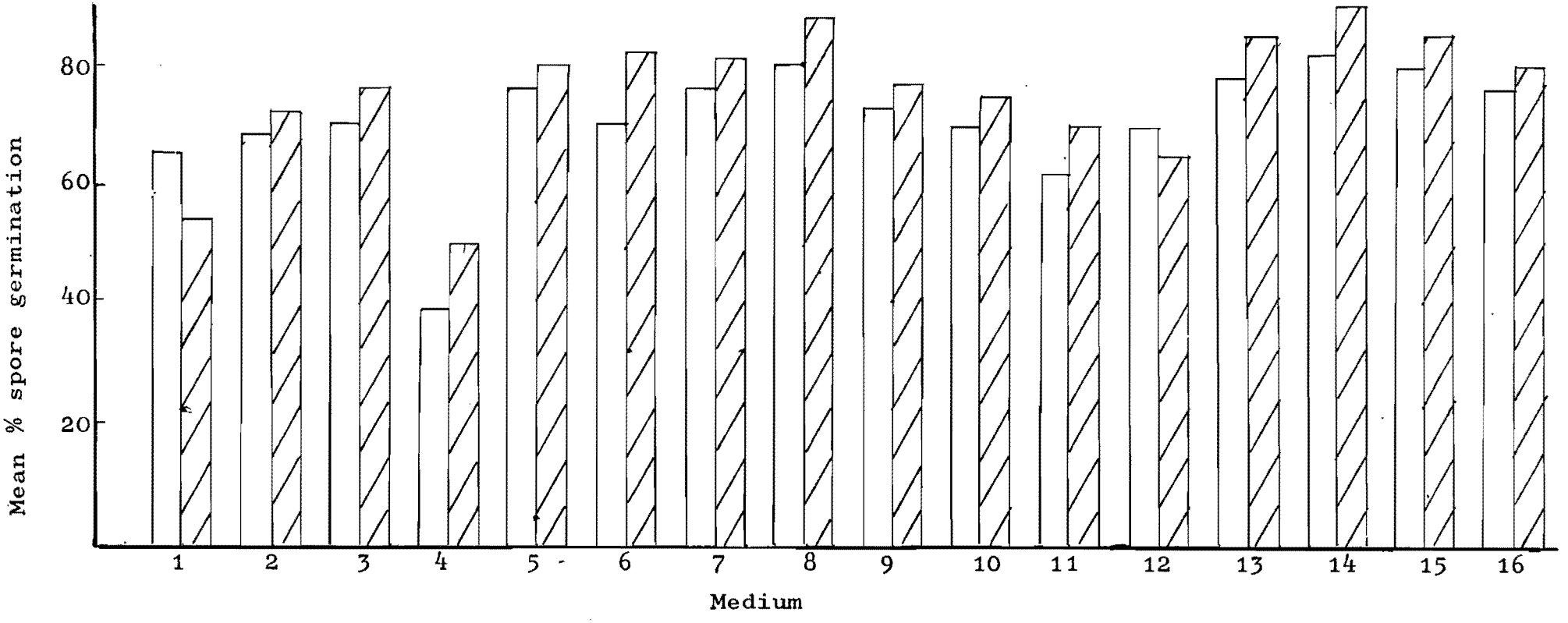


Figure 33. % germination of spore source 200 in metal and wooden (striped) incubators.

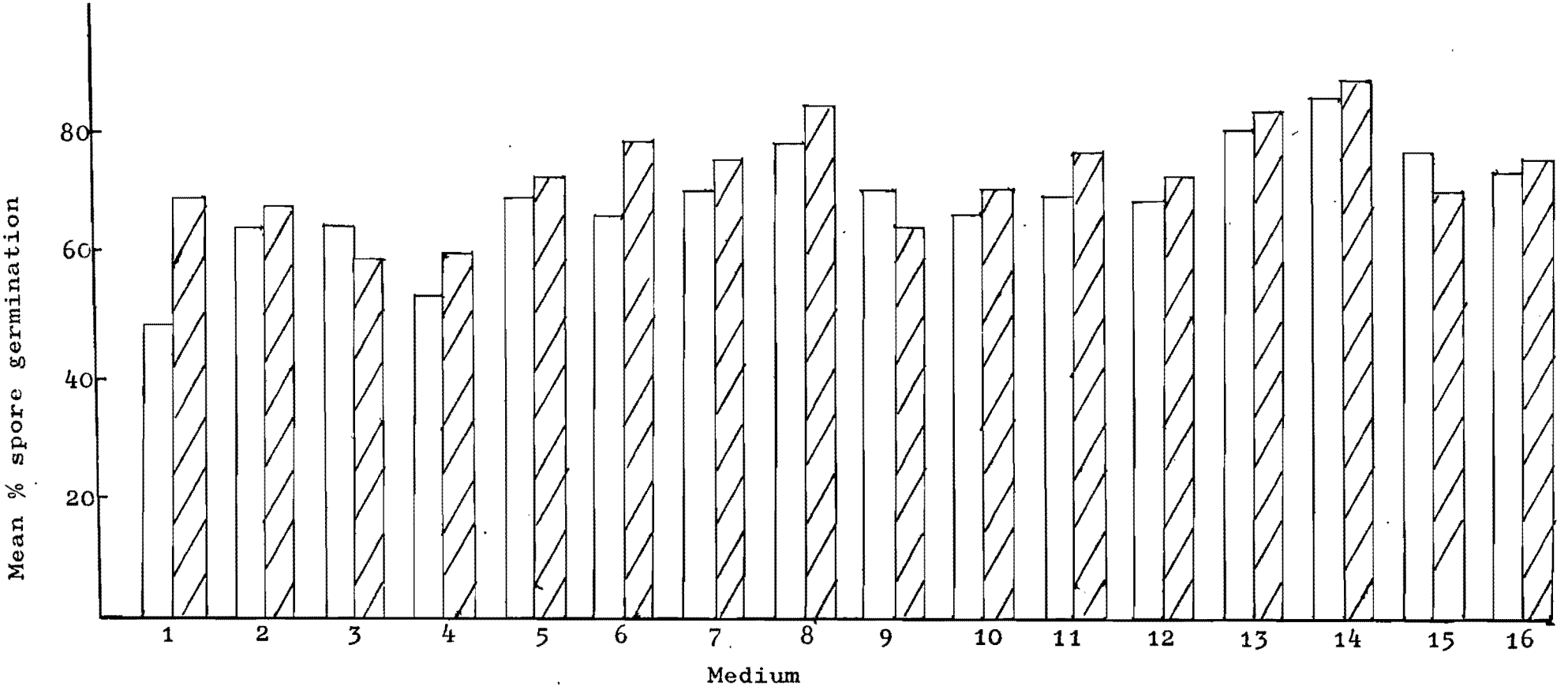


Figure 34. % germination of spore source 100 in metal and wooden (striped) incubators.

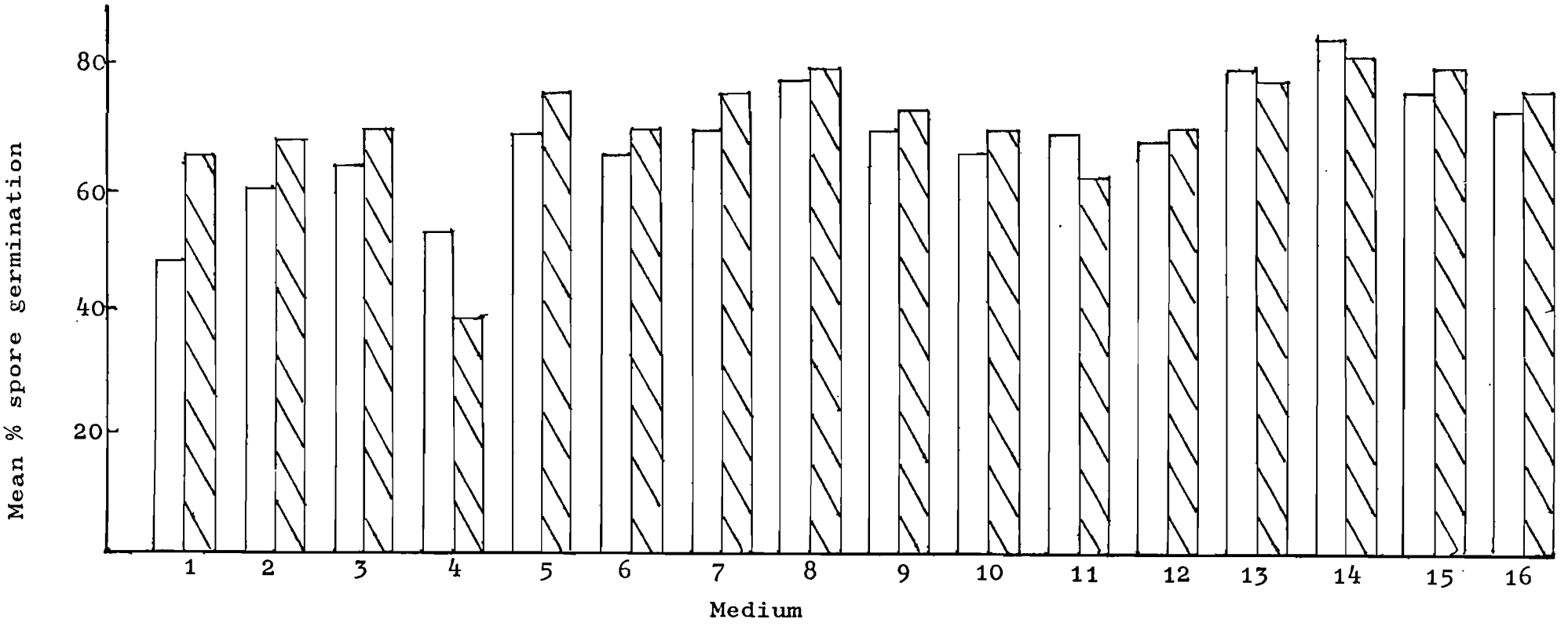


Figure 35. Percentage germination of spore sources 100 and 200 (striped) in a metal incubator.

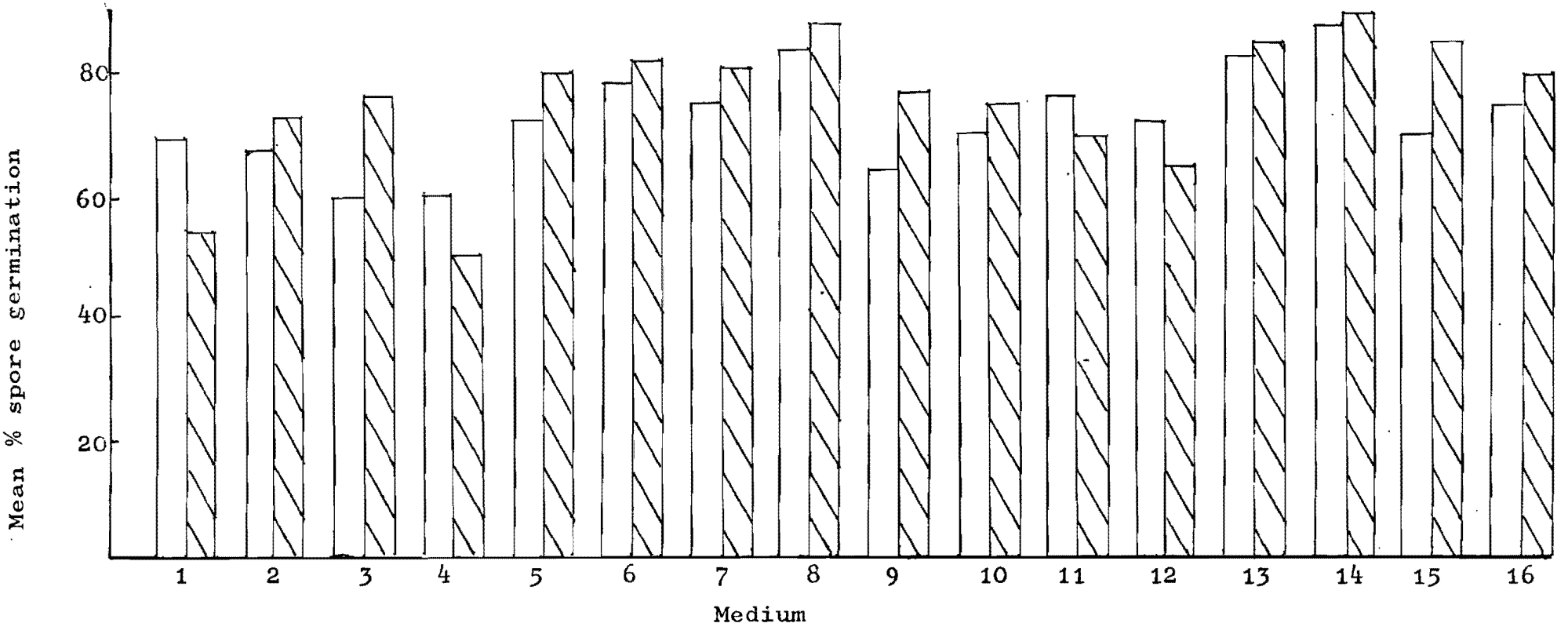


Figure 36. Percentage germination of spore sources 100 and 200 (striped) in a wooden incubator.

2. DISCUSSION

The results of this study demonstrate that most of the difficulties encountered by earlier workers to germinate Endogone spores were overcome using the media described. Germination rates were fair in water agar, but were improved considerably with the addition of calcium phytate, $CaHPO_4.2H_2O$ and inositol. The vitamins, nicotinic acid and thiamine HCl, either added separately or together proved even more favourable to spore germination. This is in agreement with the findings of Hepper and Smith (1976), who reported considerable improvement to germination rates of spores in water agar with the separate addition of nicotinic acid and thiamine HCl.

Mosse (1959) reported erratic germination of Endogone spores, which depended on special selection of spores and the provision of diffusable substances from unsterilised soil. Her experiments also incorporated time consuming procedure, in which two different types of agar were used, on which cellophane discs were placed, bearing the spores. The results of the present study show that germination of resting Endogone spores may be achieved quite simply with water agar and this germination can be enhanced with the addition of various other reagents.

Various attempts have been made to germinate Endogone spores with inconsistent results (Mosse, 1959, 1962; Mosse and Phillips, 1971) and with the necessity of a living host. Some recent work by Mosse and Hepper (1975) described attempts to germinate Endogone spores on

White's (1954) medium which were also unsuccessful. However, these latter workers established for the first time, v. a. mycorrhizal infections in clover root organ cultures, which undoubtedly offers a new dimension in the study of root:endophyte interactions.

The inability to germinate Endogone spores in previous instances may be attributed to the employment of unsuitable media. One possible reason may be the rather delicate ionic sensitivity of Endogone. For instance, high phosphorus content in the media is now widely accepted as a factor contributing to suppression of Endogone mycorrhiza development. Jensen's (1942) medium, employed by many of the workers involved in this field, is known to have a phosphorus content far in excess of that tolerable to Endogone. Hepper and Smith (1976) reported of concentrations of manganese and zinc as they occur in commercially available agars being intolerably high for Endogone spore germination. Conversely, Gilmore (1971) found that mycorrhizae formed between peach roots and the Endogone species used had enhanced the ability of the plants to obtain or utilise zinc and thus reduce the incidence of zinc deficiency symptoms. It may have been that in the latter case, Endogone growth was not suppressed by the obviously low zinc content in the soil and in turn aided in extracting zinc from less accessible sources. Results obtained by Daniels and Graham (1976) suggests that excess nutrients such as provided in standard Potato Dextrose or Nutrient Agars or those that are available subsequent to autoclaving, air-drying or fumigation of soils caused the

inhibition of germination of Endogone spores. They also concluded that certain germination factors are present as natural components in some agars but absent in more refined agars.

The observations reported in this study also indicate that not only is the selection of an appropriate medium important in attempts to germinate Endogone spores but so is the location of incubation, as shown by both the spore sources used exhibiting preference for the wooden incubator. The fact that spore source 100 showed a lower germination rate than spore source 200 indicates that the media selected here which were favourable to spore source 200 may not be so for the same or different Endogone species. Even with the same Endogone species, as was used in this study, the associated host plant's influence must be taken into consideration. In this case, most of the media used were better suited for the germination of Endogone spores which were associated with A. cepa.

Recently, a study of the germination and growth of hyphae from Endogone spores in soil was undertaken by Powell (1976) which showed that germination occured readily, with or without the presence of onion roots. This is another demonstration of the nonessentiality of the presence of a host for Endogone spore germination.

The problems met in this study will probably be amplified in any attempts to grow Endogone on a large scale. If production of Endogone on a large scale is possible, it

may make pre-inoculation of plants with the most compatible and beneficial endophyte a practical proposition.

CHAPTER X

AN ULTRASTRUCTURAL STUDY OF VESICULAR-ARBUSCULAR MYCORRHIZAL DEVELOPMENT

1. RESULTS

Extreme care taken in handling specimens during preparation of material permitted SEM examination of the interior of the onion root. The most obvious instance of damage would have been that caused by the longitudinal cut made with a razor blade. Despite this, the distribution of the v. a. endophyte within the root cortex was clearly exhibited. The treatment completely removed cytoplasm and all membrane bound organelles from exposed host cells.

Most of the root segments examined were heavily colonised by Endogone, showing abundant intracellular hyphae and vesicular and late arbuscular development. Vesicles were located in areas of extensive hyphal development (Figures 40, 41 and 42). The structure of arbuscules is clearly defined in figures 37, 38 and 39, showing their distinct dichotomous branched habit. Figures 38 and 39 show arbuscules in later stages of degeneration, where the initial collapse of terminal hyphal branches indicated in figure 37 is followed by collapse of major branches (Figure 38) and then by the collapse of the whole structure (Figure 39).

It was found that the recommended duration of exposure of root segments to KOH during the preparation

of material proved too severe and tended to not only remove host cytoplasm but also the v. a. endophyte. However, reducing the exposure time from thirty to five minutes proved adequate in reducing morphological damage.

Figure 37. Scanning electron micrograph of early arbuscular degradation, showing collapse of finer, terminal branches X 1120

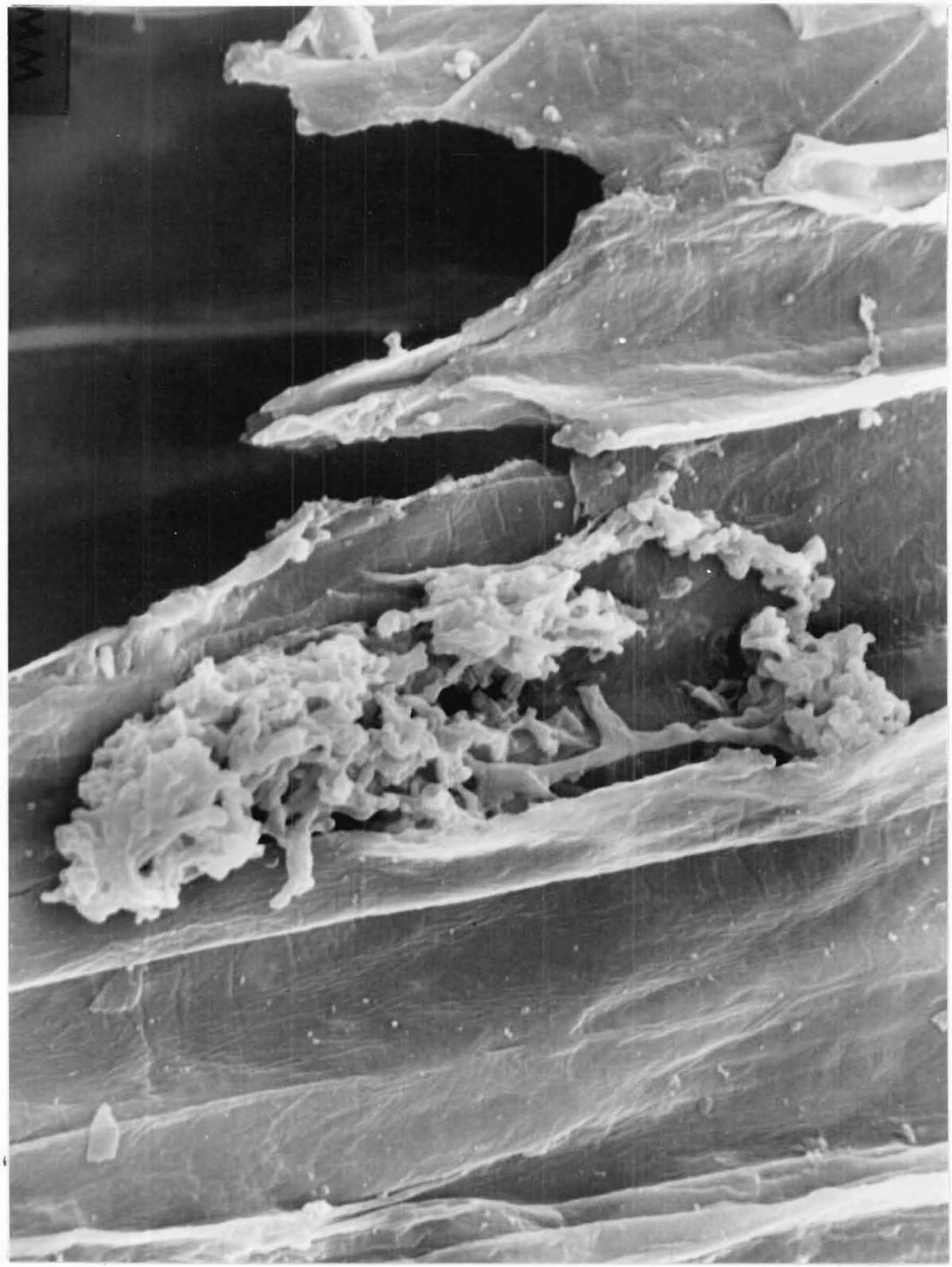


Figure 38. Scanning electron micrograph of further arbuscular degradation, showing collapse of major branches X 1120

Figure 39. Scanning electron micrograph of advanced arbuscular degradation, showing collapse of whole arbuscule X 1500

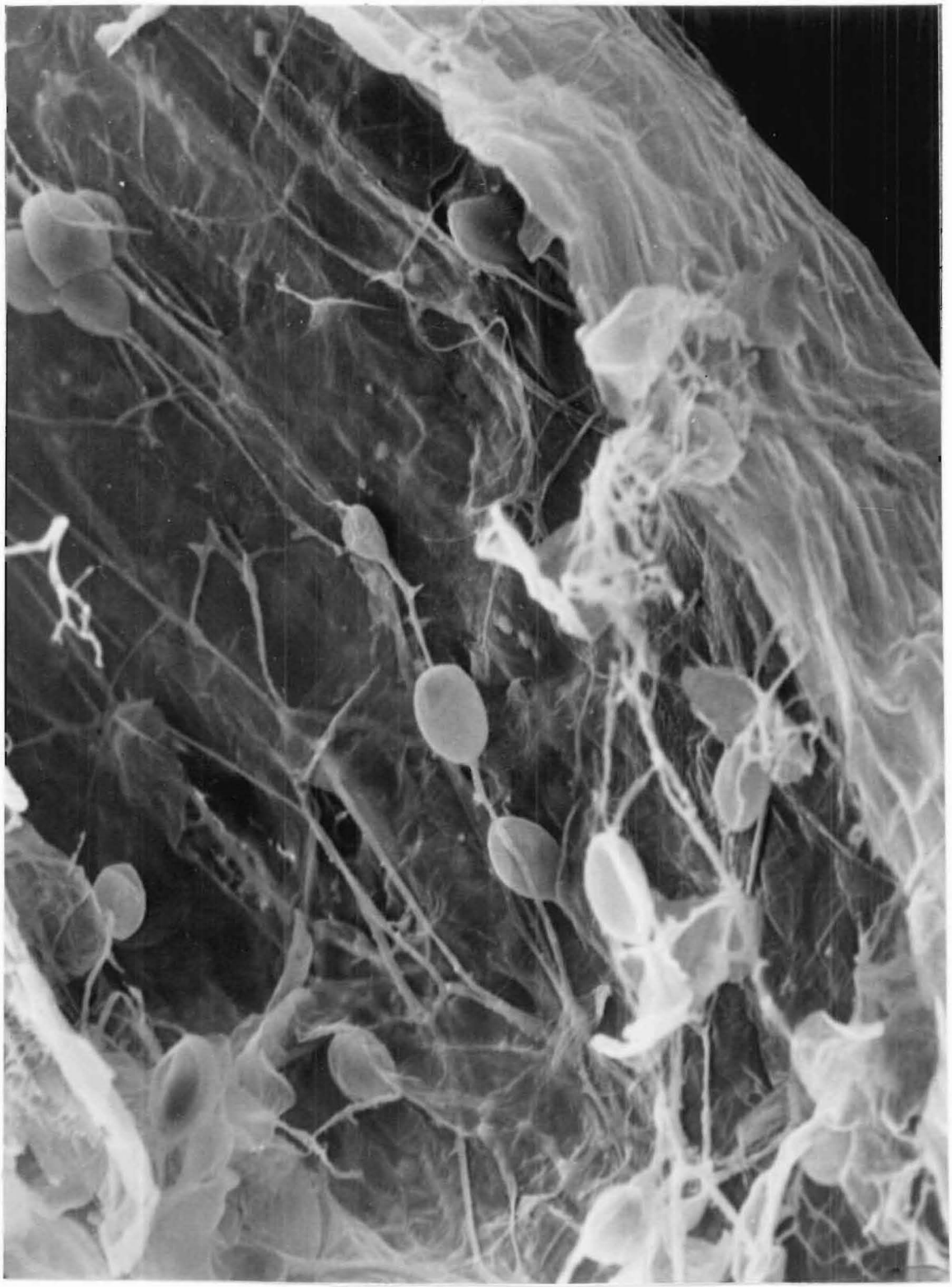


Figure 40. Scanning electron micrograph of advanced Endogone colonisation, showing abundant hyphae and vesicles X 500

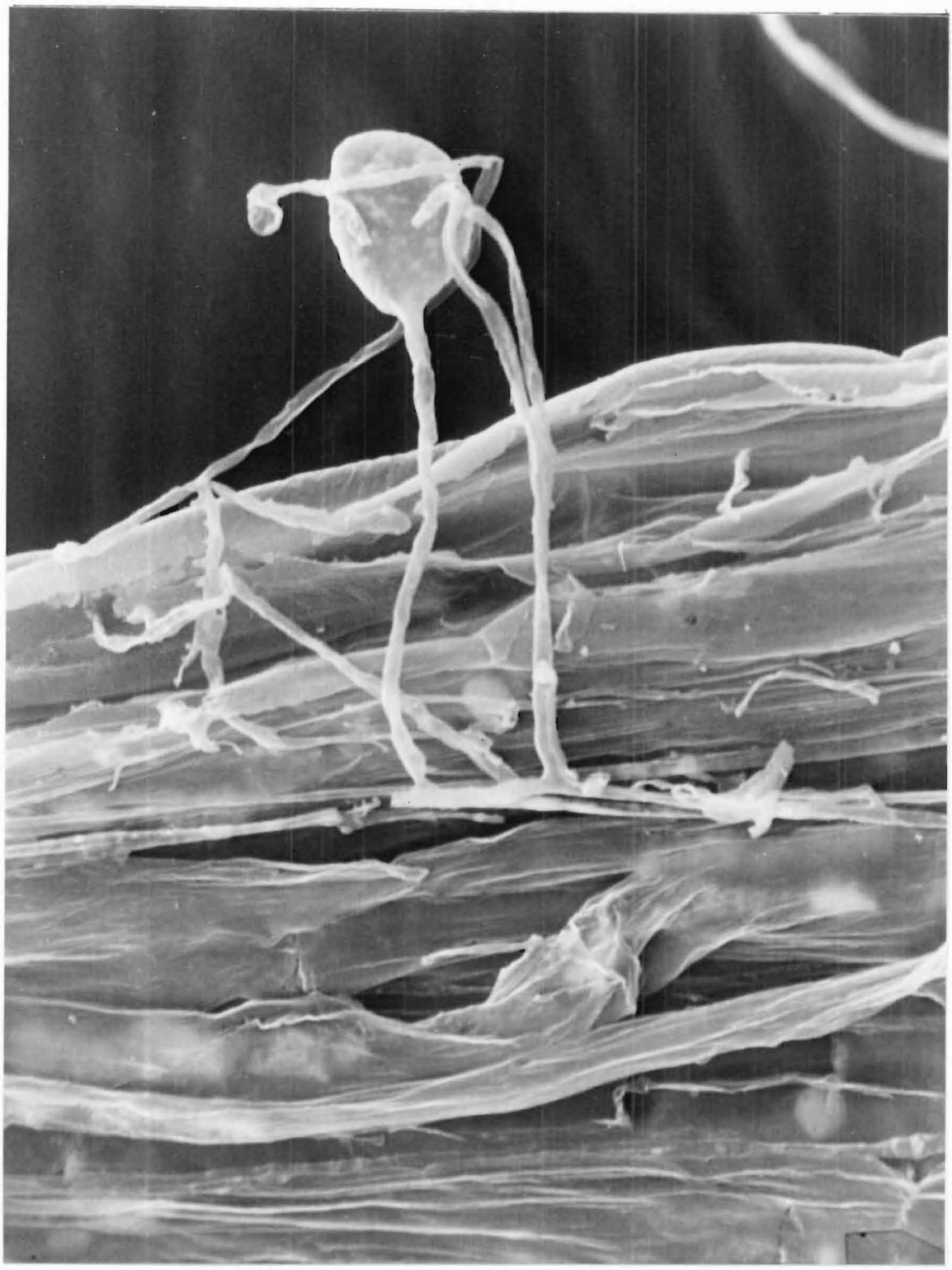


Figure 41. Scanning electron micrograph showing the relationship between hypha and vesicle X 1000

Figure 42. Scanning electron micrograph
of an Endogone vesicle,
showing finer detail
of surface of vesicle
X 2500

2. DISCUSSION

The technique employed was very successful in exposing the v. a. endophyte for examination. Detailed study of endophyte distribution was possible, especially of hyphae and vesicles. The development of arbuscules was apparently at a mature stage, as most of the arbuscules showed signs of degeneration, that is, the deterioration of arbuscular branches and subsequent accumulation of collapsed branches into clumps, which may be accompanied by aggregation of host wall material. This collapse is thought to be preceeded by solubilization of fungal cytoplasm in the arbuscules (Kinden and Brown, 1975), initiated soon after the arbuscules are formed (Cox and Sanders, 1974), during which nutrients are probably being transferred to the host (Cox and Sanders, 1974; Kinden and Brown, 1975). This contribution to the host's nutrition must be fairly substantial, considering the extensive development of arbuscules within the cortex, as indicated by the light microscopic photographs (Figures 9 and 10).

Onion root tissue was extremely delicate to handle, as shown by the need to reduce the exposure time to the clearing action of KOH from the recommended thirty minutes to five minutes. This fragility of onion root tissue was further indicated by the roots of Endogone treated plants which were resistant to F. oxysporum v. cepa and P. terrestris and which were prepared for SEM examination. Roots of these plants disintegrated during the alcohol dehydration part of the preparation of material.

It was intended to study the roots of such resistant plants to determine whether the obstruction to the fungal pathogens was of a physical nature or not. As it was, the parts of the roots which disintegrated were either restricted to root tips or almost four fifths of the length of a few roots (the proximal ends remaining). The remaining parts of the roots showed typical development of the mycorrhiza. It may be that the parts of the roots which disintegrated had not been colonised by Endogone and were infected by the fungal pathogens, which in turn lowered their ability to withstand the process of dehydration by alcohol. Although this may indicate the need for the physical presence of the v. a. mycorrhiza in order to obstruct the progress of the pathogens, it does not preclude the possibility that antibiosis may be involved.

This technique, which has permitted a closer study of intracellular structures at the SEM level, was modified by the very sophisticated critical point drying procedure introduced by Anderson (1951), which eliminates distortion-causing surface tension forces on the evaporating liquid, thus minimising drying artifacts. Kinden and Brown (1974) have suggested the use of milder oxidants such as peracetic acid or oxone to remove bound osmium in SEM preparations, thus reducing morphological damage. As they are produced now, SEM micrographs will also serve to substantiate interpretation derived from transmission electron microscopic studies.

CHAPTER XI

CONCLUDING DISCUSSION

Investigations of the rhizosphere in the last decade have resulted in the identification and isolation of several different v. a. endophytes. It is now widely accepted that the association between plant and v. a. endophyte is beneficial and sometimes essential, to the healthy growth of the host plant. Studies using different species of Endogone (Gilmore, 1971; Kleinschmidt and Gerdemann, 1972; Mosse, 1972; Mosse and Hayman, 1971) indicate that specificity exists in Endogone in that certain Endogone species are more beneficial than others. Most of the evidence accumulated showing the benefit of mycorrhizae on plants is derived from studies concerning the effect of endomycorrhizae on the uptake of nutrients, especially phosphorus. The present study confirms this beneficial effect and in addition indicates that this effect may be further extended whereby mycorrhizal plants are also provided with a means of protection against certain soil - borne fungal pathogens. Mycorrhizal orchids produce orchinol, a phytoalexin believed capable of protecting the plant from certain pathogens (Gaumann, Nuesch and Rimpan, 1960). Santoro and Casida (1962) found that some ectotrophic mycorrhizal fungi, for example, Boletus luteus, can produce antibiotics. Marx and Davey (1967) showed that pine roots are protected from Phytophthora cinnamomi by ectotrophic mycorrhizae. These are examples where plant pathogens have been suppressed by biological agents.

The study of biological control of plant diseases have been pursued by various workers (among others, Baker, 1968; Huber, Watson and Steiner, 1965; Lochhead, 1959; Mitchell, 1973; Papavizas, 1973; Sandford, 1959; Stover, 1964; Wilhelm, 1973, Zak, 1964). Relatively very few cases of applied biological control have been reported (Papavizas, 1973). Since Endogone is believed to occupy more root cortical tissue than all the other fungi put together (Gerdemann, 1968) , it may be suggested that in addition to its traditional function, Endogone may also afford defence against certain pathogens, although the mechanisms by which this may occur are not known.

In the present study, the inhibition of F. oxysporum v. cepa and P. terrestris may have been one where the presence of abundant endomycorrhizal elements presented a physical barrier to infection, or it may have been due to root exudates of Endogone treated plants favouring the growth of harmless rhizosphere organisms, thus rendering mycorrhizal roots less accessible to pathogen attack. Another means of resistance may have been the release of specific antibiotics by mycorrhizal plants in response to the presence of the pathogen. It may even have involved a more complex situation as that which may be concluded from results reported by Horton and Keen (1966, 1966a) and Keen and Horton (1965). These workers suggested that cellulase and endopolygalacturonase are important in the development of onion pink rot, incited by P. terrestris. They proposed that the synthesis of these two enzymes is regulated by an inducer:repressor mechanism. In culture,

they found that the synthesis of both cellulase and endopolygalacturonase was suppressed when the inducers, cellulose and pectin respectively, were supplemented with 0.005 M glucose. These workers concluded that high root sugar content imparts greater resistance to P. terrestris by suppressing synthesis of cellulase and endopoly - galacturonase in the pathogen. While it is unlikely that Endogone may have caused an increase of sugar content in the root by increasing its uptake, it is possible that during the degeneration of mature arbuscules, some compounds may be released whose action resemble that of an increased sugar content and thus suppress the synthesis of cellulase and endopolygalacturonase in the pathogen.

Recently, it has also been shown that mycorrhizal colonisation improves nodulation in certain legumes. Asai (1944) first indicated the influence of endomycorrhiza on nodulation in legumes, but his work has only recently been fully appreciated, as shown by the work of Crush (1974), Crush and Pattison (1975), Daft and El-Giahmi (1974) and Mosse, Powell and Hayman (1975). These workers showed that legumes inoculated with Endogone and the appropriate Rhizobium strain nodulated better than those only inoculated with Rhizobium.

Another interesting aspect of mycorrhizal infection is the strong correlation between the development of v. a. mycorrhiza and the succession of plants in dunes (Nicolson, 1960, 1963). Since v. a. mycorrhiza enhances plant growth, it is possible that in the dune habitat, mycorrhizal

infection may be a considerable additional factor influencing colonisation of sand dunes by grass species. Thus, Endogone may find another use in the stabilisation of sand or soil in the reclamation of land.

The question thus arises whether preinoculation with v. a. mycorrhizal endophytes may be a practical proposition, particularly in the growth of crop plants, in an effort to increase crop yield and possibly to protect crop plants from pathogens. Under good agricultural and horticultural conditions where nutrient levels are probably high or adequate, it would be unlikely that mycorrhizal colonisation would affect the yield of crops. On the other hand, in soils of low fertility, it may improve crop yield significantly. The possibility that v. a. mycorrhizal colonisation may provide a means of protection against soil borne pathogens could be reason enough to warrant preinoculation with Endogone.

If preinoculation of plants with Endogone is to become a practical proposition, methods must be found to produce enough inoculum for incorporation with the seed and to inoculate during the nursery stage of transplanted crops (Crush and Pattison, 1975). Because of its very specific growth requirements, attempts to culture Endogone on synthetic media have met with great difficulty. Recently, methods have been developed whereby mycorrhizal inoculum may be produced on a large scale. One of these methods is where inoculum is obtained in the form of infected roots or of soil sievings of spores and then

freeze-dried for longevity and ease of storage and handling. The work of Jackson, Franklin and Miller (1972) and Crush and Pattison (1975) have shown that such inocula can be utilised to produce mycorrhizal infection. Crush and Pattison also reported on the feasibility of seed pelleting with such inocula.

If the introduction of Endogone to plants is to have its desired beneficial effect, it would be imperative to ensure that the environment in which Endogone is to grow is such that it best supports Endogone growth. Soil analysis should be carried out to determine the levels of various ions, the most obvious one being phosphorus. Because of the increased phosphate uptake, early phosphorus toxicity may be a problem in soils already containing much phosphate. Fortunately, the mycorrhizal association is apparently self-regulatory, in that in soils already containing much phosphate, mycorrhizal colonisation probably would not develop to the stage where growth decreases due to supra-optimal phosphorus levels in mycorrhizal plants would occur under field conditions (Khan, 1972; Mosse, 1973). The concentrations of other nutrients should also be checked, especially that of manganese and zinc. Hepper and Smith (1976) found that concentrations greater than 1.36 mg. Mn/l. and 0.7 mg. Zn/l. tend to inhibit the growth of v. a. mycorrhiza. This may be significant in the establishment of mycorrhizal infection, especially when combined with the effect of water-logging and liming of soils, both of which cause significant changes in the concentration of divalent

manganese (Grasmanis and Leeper, 1966). Timonin (1946) suggested that manganese-oxidising microorganisms, which can be particularly common in the rhizosphere, may be involved in the establishment of v. a. mycorrhiza in plants.

It is interesting to note that following reports of increased uptake of phosphate by endomycorrhizal fungi in plants, some work has been stimulated concerning seed inoculation utilising phosphate-solubilising bacteria in low phosphate soils (Barea, 1969; Barea, Azcon and Hayman, 1975; Brown, 1972, 1974). However, unlike mycorrhizal fungi that establish themselves inside the plant root, such introduced bacteria need to establish themselves in competition with other microorganisms in the rhizosphere and cannot maintain a high enough population to be of significant value for plant growth. Recently, it has been shown that v. a. mycorrhizal colonisation enabled introduced phosphate-solubilising bacteria to maintain high numbers for longer periods, thus increasing their chances to influence plant growth (Barea, Azcon and Hayman, 1975). These workers also suggested a possible synergistic effect whereby such bacteria enhance phosphate uptake by Endogone from insoluble sources of phosphate.

The most widely accepted theory to account for the increased growth rates of mycorrhizal plants is that of increased phosphate uptake. These increased rates are not believed to be attributed to increased activity of the mycorrhizal roots themselves, nor to utilisation of different and/or less available sources of phosphate, but rather to the fact that the external hyphae affords an

extension to the absorption capabilities of the root system beyond the distal regions of the roots. Ultrastructural studies have further indicated that such absorbed nutrients are transferred from fungus to host cells by being released following collapse of intracellular arbuscules. Autoradiography should further elucidate the nature of such transfers.

The study of v. a. mycorrhizae and its role in plant growth and protection will undoubtedly see more progress in clarifying the finer details of mycorrhizal infection regarding both partners of the association. Most work has pointed to the beneficial effects the plant may derive from the association. There have been scattered reports by some workers (among others, Alexandrova, 1968; Harley, 1969; Khrushcheva, 1960) of the plant playing a role in the carbohydrate metabolism of the fungus. To date, little progress has been made in studying the effects the plant may have on the metabolism of the endophyte, which are obviously of significance, as the fungus is believed to be an obligate symbiont.

The next step in the study of v. a. mycorrhizae would be to obtain enough inocula of different Endogone species to effectively inoculate plants on a field scale. Such work will undoubtedly be very time consuming, but considering the effect it will have on crop yeild and possibly on plant protection, the end more than justifies the means.

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LIST OF FIGURES AND TABLES

Abbreviations used:

C. C. = Controlled conditions; F. C. = Field conditions;

S. A. = Statistical analysis; wt. = weight;

I. P. P. = In the presence of pathogens; End. = Endogone;

S. E. M. = Scanning electron micrograph; syt. = system

ADDENDA

Additional references:

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Formulae of chemical compounds used:

$CaHPO_4.2H_2O$	Calcium monohydrogen phosphate
$Ca(H_2PO_4)_2.H_2O$	Monocalcium phosphate
$C_6H_6[OPO(OH)_2]$	Inositol hexaphosphoric - acid, 28.16 % P (Phytic acid)